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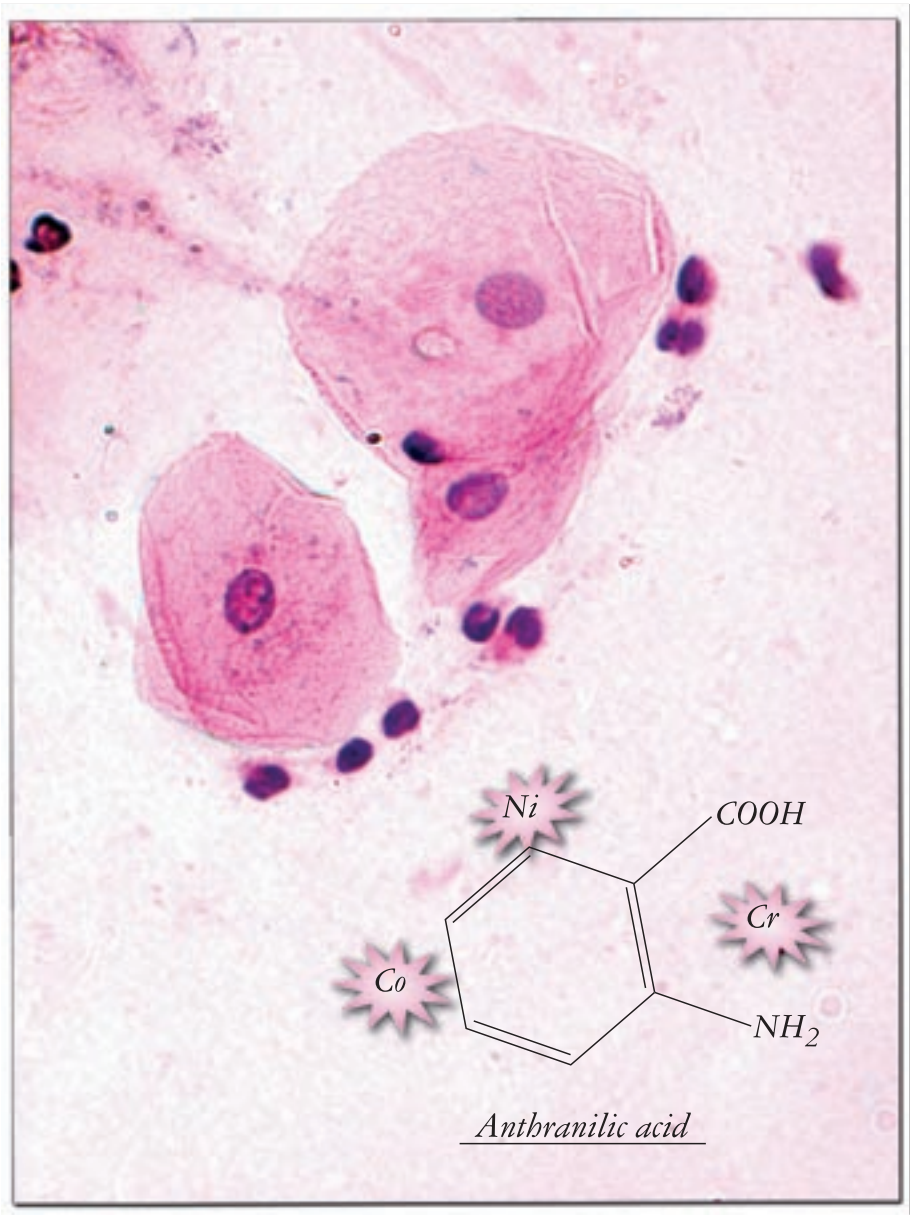

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MEDICAL UNIVERSITY *of* BIAŁYSTOK
DEPARTMENT *of* PERIODONTAL *and* ORAL MUCOSA DISEASES
DEPARTMENT *of* CONSERVATIVE DENTISTRY

Contents

Cover Illustration: The morphological picture of oral mucous epithelial cells and numerous neutrophilic granulocytes, monocytes and lymphocytes in the saliva of an allergic patient after 4 months of treatment with a fixed orthodontic appliance (Kasacka I, et al. Preliminary evaluation of saliva composition in allergic patients subjected to orthodontic treatment; morphological examination, Advances in Medical Sciences, 2006; 51, 52-4).

Clinical state of the patients with periodontitis, IL-1 polymorphism and pathogens in periodontal pocket – is there a link? (An introductory report)

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Abstract

Purpose: According to last years' research, polymorphism of IL-1 has an influence on the progression of periodontal disease. Oral mouth microflora can also have an effect on the disease process. The aim of the work was to evaluate the amount of microbacterial pathogens in the periodontal pockets of patients with positive and negative genotype.

Material and methods: Study group comprised of 16 patients, aged 25-50 years. Only patients with severe generalized form of chronic periodontititis were included into the study. After clinical examination patients were subjected to the IL-1 genotype evaluation (Genotype PST, Hain Lifescience GmbH, Germany) and PCR examination of selected bacteria pathological for periodontium (Perio-Analyse, Pierre Fabre Medicament, France).

Results: 7 out of 16 individuals were diagnosed as genotype positive (alleles 2 for genes IL-1A and IL-1B). Genetically positive individuals had greater mean pocket depth, clinical attachment loss and percentage of pockets deeper than 4 mm. Although in both groups similar bacterial pathogens have been identified, greater amounts of bacteria have been counted in group with positive genotype. Total count of bacteria from so-called "red complex" (*P. gingivalis*, *T. forsythensis*, *T. denticola*), and "orange complex" (*F. nucleatum*, *P. micros*, *P. intermedia*, *C. rectus*) were respectively 3-fold and 2-fold higher in group with positive genotype, despite the fact that group was smaller (7 vs 9 persons with negative genotype). Number and species of bacteria seems to correlate with pocket depth, clinical attachment loss, and percentage of pockets deeper than 4 mm.

Conclusion: Observed association may have an influence on increased severity of periodontal disease in patients with positive genotype.

Key words: bacteria, IL-1 genotype, periodontitis.

Introduction

The state of the periodontal diseases can be defined as an imbalance between the quality and quantity of bacterial microflora colonizing periodontal pocket, and the immunological potential of the host, which can be modified by several risk factors, among which the genotype is of greatest importance [1]. Since 1997, when professor Kornman observed 19-fold higher risk of observing bone loss in non smoking genotype-positive patients whne compared to genotype-negative [2], numerous papers confirmed the influence of IL-1 polyphormism on the outbreak and outcome of periodontal disease [3-6]. The mechanism of this linkage seems to be obvious. Rare allele of IL-1 appears to develop a susceptible phenotype, in which during inflammatory response macrophages secrete greater amounts of this cytokine, which effect is an increased inflammatory response. This results in greater damage of periodontal tissues. In in vitro studies there seemed to be a straight Mendelian correlation between IL-1 genotype and the amount of cytokine secreted by cultured macrophages [7]. However, it was not confirmed in in vivo studies. This may be caused by the enormous number of factors additionally affecting IL-1 secretion in real periodontal pocket environment.

Bacterial pathogens obviously influence the amount of IL-1 secreted by macrophages. It is well known, that Gram-negative anaerobic rods are regarded as the major pathological factor in chronic periodontitis [8-10]. In physiological conditions, low concentrations of periodontal pathogens constantly colonizing gingival sulcus are kept in check by an intact immune system.

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However, if the defence system is impaired by a genetic predisposition (interleukin-1 polymorphism), medication or smoking, the bacteria can proliferate freely leading to the manifestation of profound periodontitis. In dental plaque biofilm microorganisms form very complex structure of co-dependencies, creating specific multicellular scaffold, which sometimes express different features than isolated bacteria forming it. Therefore it is rather inaccurate to evaluate in vitro influence of dental plaque on periodontal tissues. Socransky et al. proposed the simplified model of bacterial biofilm in periodontal pockets. He divided microorganisms forming dental plaque into four complexes, from which so-called "red complex", consisted of obligatory anaerobic black-pigmented bacterial species, including *P. gingivalis*, *T. forsythensis* and *T. denticola*, seems to be mainly responsible for the severity of periodontal disease [11]. Bacteria from this group provoke increased non-specific inflammatory response. They are characterized by the production of various metabolites leading either to the direct destruction of the surrounding periodontal tissue or the activation of non-specific host defence reaction.

IL-1 plays a key role in this phase of host reaction, stimulating directly or through matrix metaloproteinases/PGE_2 resorption of connective tissue and bone. It seems reasonable to expect the influence of the bacteria present in dental plaque on the IL-1 expression determined by the host genotype. Therefore the aim of the authors was to evaluate the microbiology of periodontal pocket with regard to the host genotype and clinical examination of the patients with chronic periodontitis.

Material and methods

Study group was assembled from 16 individuals, aged 25-50 years, attending to the Department of Periodontology and Oral Diseases. Clinical examination included recording of standard periodontal parameters: pocket depth (PD), clinical attachment loss (CAL), simplified bleeding index according to Ainamo&Bay (BI) and simplified plaque index according to O'Leary (PI). Only patients with severe generalized form of chronic periodontitis were included into the study. Criteria of selection, apart from those defining the diagnose (>30% of sites with CAL, at least one site with CAL>4 mm), were as follows:

- age between 25 and 50 years
- no smoking or ceased smoking more than 2 yrs ago
- no periodontal treatment in recent 12 months
- no less than 20 teeth, and at least 3 teeth in each quadrant
- at least 2 teeth with PD>6mm, not in the same quadrant
- no antibiotic taken in recent month.

After clinical examination patients were subjected to the IL-1 genotype evaluation. This was performed with ready-to-use sets provided by the manufacturer (Genotype PST, Hain Lifescience GmbH, Germany). Sample of epithelial cells from buccal mucosa was collected using sterile foam swab. The buccal mucous membrane was dried with the dental air syringe and isolated from saliva flow. Then the epithelial surface was rubbed for 20-30 seconds with the swab, after which it was drying for about 1 minute and placed in hermetic transport tube and sent to the manufacturer's laboratory for genetic examination. Within the IL-1 gene cluster polymorphisms of IL-1α gene (IL-1A) and IL-1β gene (IL-1B) (located at -889 and at +3953 of 2nd chromosome, respectively), show a close association with periodontitis. Within both polymorphisms allele 1 harbors a cytidin (C), whereas allele 2 carries a thymidin (T) at the respective position. In particular, when both genes carry allele 2 a strong over-production of interleukin-1 will occur. Therefore an individual was regarded as "genotype-positive", if in both IL-1A and IL-1B loci alleles 2 were identified, in the same manner as described by Kornman et al. [2].

Table 1. **Mean clinical parameters in study group divided according to the result of the genetic test**

	PD [mm]	CAL [mm]	BI [%]	PI [%]	PD>4 [%]
Positive (n=7)	5.1	6.5	31.3	48.2	23.8
Negative (n=9)	3.5	4.2	38.0	62.4	11.1
Stat. signif.	p<0.05	p<0.05	N.S.	N.S.	p<0.05

PD – pocket depth; CAL – clinical attachment loss; BI – bleeding index; PI – plaque index; PD>4 – pockets deeper than 4 mm

Microbiological examination of periodontal pockets was performed using PCR reaction, which takes place on the level of the nucleic acids and therefore does not require any viable organism. Due to the high specificity of the PCR, any potential contamination of the probe by concomitant flora has no influence on the test result. Again, for this examination ready-to-use kits were used (Perio-Analyse, Pierre Fabre Medicament, France). Samples were collected from two deep pockets (PD>6 mm), and two shallow pockets (PD<3 mm) of each individual. Sampling was performed before any mechanical debridement included in treatment plan. Prior to sampling, the supra-gingival plaque was gently removed and the site of sampling was dried with a sterile cotton roll. Using a pair of sterile forceps one paper point at a time was inserted into the pre-defined sites down to the base of the sulcus and left in that position for 10 seconds. Afterwards it was transferred to the respective transfer tube and sent to the manufacturer for evaluation. Perio-Analyse test evaluates quantity of 9 pathogens: *Porphyromonas gingivalis*, *Prevotella intermedia*, *Eikenella corrodens*, *Campylobacter rectus*; *Actinobacillus actinomycetecomitans*, *Fusobacterium nucleatum*, *Tannerela forsythensis*, *Peptostreptococcus micros* and *Treponema denticola*. Titers higher than $1x10^5$ were detectable.

Results were subjected to statistical analysis with the use of modified t-Student test and Pearson correlation test. Differences and correlations were regarded as statistically significant if p index was reaching value below 0.05

Results

In study group 7 individuals were discovered to be genetically positive, 9 individuals had negative genotype (not shown). *Tab. 1* presents mean values of examined clinical parameters when group is divided according to the results of genetic test. In

***Table 2*. Results of microbiological evaluation in study group divided according to the results of genetic test**

Bacteria (x10^5)	A.a	T.f	C.r	T.d	E.c	P.i	P.m	P.g	F.n	White	Light grey	Grey
Positive (n=7)	286.3	1855.6	406.9	946.4	177.1	87.1	1377.6	2604.6	2754.3	5406.6	4625.8	463.5
Negative (n=9)	43.6	741.1	114.7	761.3	62.2	355.9	1309.2	252.6	926.6	1755.0	2706.3	105.8
Stat. signif.	N.S.	N.S.	$p<0.05$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

A.a – *Actinobacillus actinomycetemcomitans*; T.f – *Tanerella forsythensis*; C.r – *Campylobacter rectus*; T.d – *Treponema denticola*; E.c – *Eikenella corrodens*; P.i – *Prevotella intermedia*; P.m – *Peptostreptococcus micros*; P.g – *Porphyromonas gingivalis*; F.n – *Fusobacterium nucleatum*; White – Total number of red complex bacteria; Light grey – total number of orange complex bacteria; Grey – total number of blue complex bacteria

***Table 3*. Evaluation of Pearson correlation in study group**

	PD	**CAL**	**BI**	**PI**	**PD>4**
Red	0.52	0.51	0.43	0.06	**0.66**
Orange	0.16	0.45	0.42	0.07	0.42
PD	x	**0.79**	0.32	0.07	**0.85**
CAL	**0.79**	x	0.42	0.17	**0.77**
BI	0.32	0.42	x	**0.74**	0.53
PI	0.07	0.17	**0.74**	x	0.25
PD>4	**0.85**	**0.77**	0.53	0.25	x

PD – pocket depth; CAL – clinical attachment loss; BI – bleeding index; PI – plaque index; PD>4 – pockets deeper than 4 mm; Red – total number of red complex bacteria

genetically positive group there was a higher mean pocket depth around the tooth with most advanced periodontal disease (one of the sites studied microbiologically), as well as higher mean clinical attachment loss and higher mean percentage of pockets deeper than 4 millimeters, though plaque index is slightly lower. Bleeding index in both subgroups is almost at the same level.

Tab. 2 presents mean titers (x 10^5) of bacteria from probed sites of patients genetically positive and negative. As seen here, there are differences between both subgroups when evaluating all the species studied. Only *Prevotella intermedia* mean titer was higher in group with negative result of genetic test. Total number of *Campylobacter rectus* species in genetically positive group was significantly higher ($p<0.05$). Other microorganisms were also present in greater numbers in the subgroup genetically susceptible to periodontitis, though the difference was not statistically significant. More evident results are obtained when evaluating complexes of bacteria. All three complexes studied were detected in higher numbers in group of patients with positive genotype ($p>0.05$).

Next table shows the initial results of Pearson's correlation. There is a statistically significant positive association between PD and CAL, between both PD and CAL and percentage of PD deeper than 4 millimeters, and between plaque and bleeding indices. There also was positive correlation between red complex bacteria and percentage of PD deeper than 4 millimeters. Significant correlation was also found when evaluating bacteria species: there was a positive correlation between *Porphyromonas gingivalis* and PD/PD>4 (values 0.58 and 0.67, respectively), *Campylobacter rectus* and PD/CAL/PD>4 (values 0.63, 0.69 and 0.71, respectively; data not shown).

Discussion

Though not without critical opinions [12-14], genetic factor remains a reliable predictor of the outcome of periodontal disease. Kornman et al. observed among non-smokers suffering from periodontitis 18,9-fold greater risk of bone loss [2]. Reports of other authors are discussing the correlation between genotype and clinical markers of periodontal disease. According to Shapira et al., who searched the Medline-PubMed for the articles wrote from january 2000 up to and including march 2005. Genetic variability was examined for the correlation to clinical indicators of inflammation such as bleeding on probing (BOP), gingival inflammation, cytokine in gingival crevicular fluid (GCF) and cytokine production by inflammatory cells. Authors found no correlation between any of the gene polymorphisms and mentioned clinical indicators of inflammation [15]. Also Konig et al. in a study performed on 53 non-smokers did not observe association between pocket depth and IL-1 genotype [16]. On the other hand, Cullinan et al. in the longitudinal study on 295 non-smoking subjects observed a relationship between the IL-1 positive genotype and increased mean probing pocket depth in non-smokers aged above 50 [17]. Also Thomson and coworkers observed in a New Zealand population 12,3-fold greater risk of being classified to "severe" group when genetically positive, while "severe" was defined as more than one teeth with PD greater than 5 mm [18]. In our study group, though clinically appear similar, genetically positive patients turn out to have deeper pockets in greater number of sites, despite the lower number of tooth surfaces covered with plaque. It seems reasonable to assume, that not only the similar amount of bacteria stimulate greater production of IL-1, but also the shift in the quality of microflora should be observed. Own observation supports this thesis and stays in agreement with the report of Socransky et al. [19], who in 108 systemically healthy subjects with refractory periodontitis observed over-representation of red and orange complex bacteria in the subgroup of genetically positive patients.

Other authors' reports support also the initial correlation results. Kawada and coworkers found a significant positive correlation between the number of *Porphyromonas gingivalis* and PD. The slope of the regression line indicated that for every 1-mm increase in pocket depth, the number of *Porphyromonas gingivalis* increased 10-fold [20]. Klein and Goncalves observed association between prevalence of *Tanerella forsythensis* in a subgingival sampling and the incidence of pocket deepening and clinical attachment loss [21].

Since individuals creating study group were selected

amongst the patients of the Department of Periodontology, the predictability of the genetic test is of no practical use. As periodontists-practitioners, authors can only state the presence of periodontal disease and conduct a proper treatment. Although the reports about the influence of genotype on the treatment outcome suggest bad or at least unpredictable prognosis [6], information about being genetically susceptible was of great value for studied subjects. Since 4 of them are parents, they are aware that their children may inherit the predisposition for periodontitis, and will put greater attention on a proper prophylaxis.

Since the number of subjects included is still very low, it is very risky to draw any far-going conclusions. As for now, it can be stated that:

1) Initial results show a significant difference in clinical parameters between genetically positive and negative patients. Individuals with alleles 2 on both loci coding IL-1 had greater mean pocket depth, clinical attachment loss and greater percentage of pockets deeper than 4 mm;
2) There seems to be a relationship between genotype and the environment of subgingival microflora. Genetically positive patients not only have 3 times more bacteria from red complex and 2 times more bacteria from orange complex than patients with negative genotype, but there are also differences when evaluating single bacterial species, particularly *Porphyromonas gingivalis* (10-fold higher titers in genetically positive individuals);
3) There is a positive correlation between red complex bacteria and percentage of pockets deeper than 4 millimeters (PD>4), between PD, CAL and PD>4, PI and BI.

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Polymorphism in interleukin-1β gene and the risk of periodontitis in a Polish population

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Abstract

Purpose: The aim of the present study was to explore an association between IL-1B polymorphism and periodontal disease in patients with chronic periodontitis and subjects with aggressive periodontitis in a Polish population. In multivariate logistic regression the association of the following parameters: genotype, age, sex, smoking status, and approximal space plaque index (API) >50% with the risk of periodontitis was analyzed.

Material and methods: Fifty-two unrelated patients suffering from periodontitis, 20 of them with generalized aggressive periodontitis and 32 with generalized advanced chronic periodontitis were enrolled into the study. Control group consisted of 52 healthy volunteers, without signs of periodontitis. IL-1B^{+3954} polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: There were no significant differences in the distribution of IL-1B^{+3954} genotypes and alleles between periodontal patients either with chronic or aggressive periodontitis and the controls. A predisposing genotype consisting of allele 2 was carried by 34.4% of subjects with chronic periodontitis, 25.0% of subjects with aggressive periodontitis, and 40.3% of healthy subjects. Multivariate logistic regression analysis revealed significant association of age ($p=0.003$), smoking ($p=0.03$), and API >50% ($p=0.002$) with the appearance of aggressive periodontitis, as well as API >50% ($p<0.001$) with chronic periodontitis.

Conclusions: The study revealed no association of IL-1B polymorphism and the risk of aggressive and chronic periodontitis. The risk of aggressive periodontitis was significantly associated with age, smoking, and oral hygiene where as chronic periodontitis with oral hygiene only.

Key words: polymorphism, interleukin-1B gene, periodontitis.

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Introduction

Periodontitis is a chronic disease of the tooth-supporting tissues which is characterized by gingival inflammation and alveolar bone loss. Although oral bacterial infection is a major factor of periodontitis, its progression and severity depends upon interplay between genetic and environmental factors. Therefore, there have been numerous attempts to define genetic factors implicated in periodontal diseases and to establish an association between candidate genes and severity of periodontitis. Since the cross-sectional study published by Kornman [1], one of the most studied genetic association with periodontal diseases is that of interleukin-1 (IL-1) genotype. IL-1 is of special interest in the context of periodontitis due to its modulating role in synthesis and resorption of extracellular matrix components and bone of the periodontal tissues [2]. Increased levels of IL-1β have been found in both gingival crevicular fluid [3] and gingival tissues [4] of patients with chronic (adult) periodontitis. Variation in cytokine levels among individuals may contribute to the disease susceptibility [5], and may be attributed in part to particular alleles of polymorphic IL-1 gene [6]. A cluster of genes regulating production of the pro-inflammatory cytokine consists of IL-1A, IL-1B and IL-Ra gene, encoding IL-1α, IL-1β and IL-1ra (receptor antagonist) respectively, and lies on the long arm of chromosome 2. Bi-allelic polymorphism in the 5th exon, position +3954 of the IL-1B have been described [7]. Allele 2 of IL-1B gene was related to increased production

Table 1. **Clinical parameters of patients with periodontitis and with healthy periodontium**

Clinical parameters*	Aggressive periodontitis (AgP) (n=20)	Chronic periodontitis (CP) (n=32)	Healthy periodontium (HP) (n=52)
API (%)†	53.7 ± 9.0	81.8 ± 19.0	34.3 ± 10.3
mSBI (%)†	76.6 ± 19.9	78.0 ± 15.0	2.1 ± 3.1
PPD (mm)†	7.99 ± 0.79	6.35 ± 0.40	1.49 ± 0.30
CAL (mm)†	7.73 ± 0.70	7.38 ± 0.70	0.06 ± 0.18
Mobility (Periotest)	17.3 ± 7.2	21.0 ± 5.8	2.7 ± 1.7

* API – approximal plaque index; mSBI – modified sulcus bleeding index; PPD – probing pocket depth; CAL – clinical attachment level; †mean values ± SD; p-Value <0.01

of IL-1β in vitro [7] and by peripheral blood leukocytes [8]. Heterozygous individuals for the IL-1B allele 2 produce twice as much IL-1β, while homozygosity promotes a 4-fold increase in the production of IL-1β [8]. Kornman et al. [1] identified a composite genotype, linked to severity of chronic (adult) periodontitis in non-smokers. Several other studies have tried to correlate various periodontal conditions with either presence of the composite genotype or one of the alleles.

No data exists in the medical literature concerning the frequency of IL-1B gene polymorphism in Polish subjects diagnosed with periodontitis. Therefore, the aim of the present study was to explore an association between IL-1B genotype and periodontal disease in patients with generalized advanced chronic periodontitis and patients with generalized aggressive periodontitis.

Materials and methods

Subject sample

Within the protocol approved by the Ethics Committee of Pomeranian Medical University, Szczecin, Poland, subjects signed informed consent. A total of 52 unrelated patients suffering from periodontitis (29 females, 23 males), aged 22-60 years (mean 41.9 ± 8.9 years), recruited from patients presenting at the Department of Periodontology, Pomeranian Medical University, Poland, were enrolled into the study. All patients, in good general health, were examined by the same investigator using a manual Williams probe (Hu-Friedy). Diagnosis of periodontal disease was made on the basis of clinical parameters and radiographic examination: approximal space plaque index (API) [9], modified sulcus bleeding index (mSBI) [10], probing pocket depth (PPD), clinical attachment level (CAL). Measurements of probing depth and attachment level were recorded at six sites per tooth, and the greatest value for each tooth was used in statistical analyses. Assessment of tooth mobility was performed with the use of Periotest instrument (Siemens AG, Bensheim, Germany) [11]. Based on the criteria proposed by International World Workshop for a Classification of Periodontal Diseases and Conditions [12], the patients were assigned into the groups containing 20 subjects with generalized form of aggressive periodontitis (AgP) and 32 with generalized advanced chronic periodontitis (CP). Control group (HP) consisted of 52 healthy volunteers (34 females, 18 males) aged 22-60 years (mean 41.6 ± 9.8 years), free from signs of periodontitis. Clinical parameters of the study population are summarized in *Tab. 1*. In all subjects, both with periodontitis and healthy ones, smoking status was classified as current smoker, non-smoker (never smoke), and former smoker.

DNA extraction and analysis of the IL-1B genotypes

Genomic DNA came from leukocytes contained in 450 μl of venous blood with ethylene diamine tetra-acetic acid as an anticoagulant. DNA was then precipitated in 95% ethanol, dissolved in distilled water and stored at -20°C until analysis. IL-1B^{+3954} polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Specific oligonucleotide primer pair [1]: 5'-CTC AGG TGT CCT CGA AGA AAT CAA A-3' and 5'-GCT TTT TTG CTG TGA GTC CCG-3' (2 μM) was used. The lower master mix contained: 1x PCR buffer (10 mM Tris-HCl [pH 8.8], 1.5 mM $MgCl_2$ 50 Mm KCl, 0.1% Triton X-100); 1mM $MgCl_2$; 0.2 Mm dNTPs; 2.0 U Taq1 polymerase (Gibco BRL Life Technologies, Glasgow, Scotland).

Thermocycling conditions: 2 cycles of denaturation at 95°C for 2 min, annealing of primers at 67.5°C for 1 min and extension at 74°C for 1 min was followed by 35 cycles at 95°C for 1 min, 67.5°C for 1 min and 74°C for 1 min. Finally, 3 cycles of 95°C for 1 min, 67.5°C for 1 min and 74°C for 5 min completed the cycling. The amplified DNA was digested with Taq1 at 65°C for 3 h. The resulting products of 12bp+85bp+97bp (allele 1) and 12bp+182bp (allele 2) were then subjected to electrophoresis on 4% agarose gels.

Statistical analysis

Allelic and genotype frequencies are presented as odds ratios (ORs) and 95% confidence intervals (CIs). The χ^2 or the Fisher's exact tests were used for analysis of allelic prevalence, genotypes, and for a deviation of genotype distribution from the Hardy-Weinberg equilibrium. In multivariate logistic regression the following parameters: genotype, age, sex, current smoking, smoking habits and API >50% were analyzed.

Results

Prevalence of genotypes and alleles in periodontitis patients and controls is presented in *Tab. 2*. There were no significant

***Table 2*. Distribution of patients with periodontitis and with healthy periodontium according to IL-1B genotypes**

	Aggressive periodontitis (AgP) (n=20)		Chronic periodontitis (CP) (n=32)		Periodontitis (P) (n=52)		Healthy periodontium (HP) (n=52)	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Genotype								
1/1	15	75.0 (45-94)	21	65.6 (48-79)	36	69.2 (56-80)	31	59.6 (46-72)
1/2	5	25.0 (11-47)	11	34.4 (20-51)	16	30.8 (20-44)	19	36.5 (25-50)
2/2	0	0	0	0	0	0	2	3.8 (1-13)
Allele								
1	35	87.5 (74- 94)	53	82.8 (72-90)	88	84.6 (76-90)	81	77.9 (69-85)
2	5	12.5 (5-26)	11	17.2 (9-28)	16	15.4 (20-23)	23	22.1 (15-31)

* lack of statistical differences AgP vs HP, CP vs HP, P vs HP

***Table 3*. Univariate and multivariate logistic regression analysis**

Parameters	Aggressive periodontitis (AgP) n=20		Chronic periodontits (CP) n=32		Periodontitis (P) n=52	
	univariate regression OR (95%CI)	[1]multivariate regression OR (95%CI)	univariate regression OR (95%CI)	[1]multivariate regression OR (95%CI)	univariate regression OR (95%CI)	[1]multivariate regression OR (95%CI)
IL-1B 1/2+2/2 vs 1/1	0.49 (0.15-1.59)	0.35 (0.05-2.38)	0.77 (0.30-1.95)	0.80 (0.15-4.36)	0.66 (0.29-1.49)	0.78 (0.21-2.84)
age		0.83 (0.73-0.94)*		1.02 (0.91-1.13)		0.93 (0.86-1.00)
sex		1.77 (0.34-9.06)		0.80 (0.14-4.47)		1.16 (0.34-3.91)
current smoking		0.10 (0.002-4.41)		0.18 (0.005-6.14)		0.10 (0.004-2.28)
smoking (former and/or current)		12.43 (1.27-121.9)*		4.59 (0.30-70.40)		10.00 (1.37-72.72)*
API > 50%		25.32 (3.08-208.0)*		79.60 (14.35-441.53)*		52.94 (12.44-225.38)*

* statistical significance $p<0.05$; [1] statistical significance for multivariate analysis model: $p<0.001$

differences in the distribution of IL-1B^{+3954} genotypes and alleles between the periodontal patients (P) and the controls (HP). A predisposing genotype consisting of allele 2 was carried by 34.4% of subjects with chronic periodontitis, 25.0% of subjects with aggressive periodontitis, and 40.3% of healthy subjects. Genotype consisting of two IL-1B^{+3954} allele 1 more frequently occurred in patients with AgP compared to CP as well as to healthy subjects.

Since smoking has been recognized as a strong confounding factor of periodontitis, the non-smokers were also considered separately. Genotypes including the IL-1B allele 2 (1/2, 2/2) were observed in 50.0% of patients with chronic periodontitis, 36.5% of control subjects and only in 22.2% of patients with aggressive periodontitis. However, these differences failed to reach statistical significance.

Univariate logistic regression (*Tab. 3*) analysis indicated no association between the studied genotype and the periodontal disease, neither aggressive nor chronic. Multivariate logistic regression revealed significant association of age ($p=0.003$), smoking ($p=0.03$), and approximal space plaque index >50% ($p=0.002$) with the appearance of AgP, as well as API >50% ($p<0.001$) with CP. When all patients with periodontitis were considered, significant association between the periodontal diseases and smoking ($p=0.02$) as well as API >50% ($p<0.001$) was revealed.

Discussion

The impact of genetic factors on various forms of periodontal disease has been incorporated into periodontology as a new line of research. Having in mind recent scientific advances, pathogenesis of periodontitis can be considered as an interplay between environmental and genetic factors. Of the latter, one of the best established is IL-1B gene polymorphism. Since the pioneering paper of Kornman et al. [1] published in 1997 pointing out the role of IL-1 polymorphism in severity of periodontitis, other authors have significantly contributed to our understanding of nature of IL-1. There have been published data supporting the initial observations of Kornman et al. on the genes encoding the cytokines IL-1α and IL-1β, alone and in combination, and prevalence and/or severity of chronic periodontitis [1,8,13-16]. However, it should be noted that these prevalence studies including controls refer to populations of moderate sample size. McDevitt et al. [17] demonstrated higher, although statistically not significant, prevalence of combined IL-1 genotype (allele 2 for both IL-1A and IL-1B genes) in patients with moderate to severe periodontitis (41%) in comparison to healthy subjects and patients with mild periodontitis (28%). Similar results were published by Laine et al. [15] (56.6% vs 41.5% in controls). Interesting data were presented by Parkhil et al. [18]. The authors observed predominance of subject characterized by

allele 1 of IL-1B gene among patients with aggressive periodontitis (early onset periodontitis). This finding may suggest various effects of IL-1B gene polymorphism in patients with aggressive and chronic periodontitis. However, it should be noted that there are no well established data whether IL-1 secretion solely depends on its genotype. Mutual influence from other cytokines is also present but no information of their genetic background on IL-1 secretion is available. IL-1B genotype may exert clinical effects on periodontitis occurrence and course as a part of complex interplay with other genes implicated in pathogenesis of the disease. IL-1B gene is positioned on chromosome 2 next to other IL-1 family genes or their receptors. Therefore, the defined haplotypes may be associated with aggressive periodontitis (early onset periodontitis), e.g. allele 1 of IL-1B^{+3954} and allele 1 of IL-Ra [18]. Similar findings were reported by Diehl et al. [19], who observed significant prevalence of allele 1 of both IL-1A and IL-1B genes in families with at least two patients diagnosed with aggressive (early onset) periodontitis.

In the present study, involving Polish patients, diagnosed with periodontitis, both aggresive and chronic, no association of IL-1B genotype and the disease was documented. In the studied periodontitis population IL-1B genotype 1/1 was found in 69.2%, heterozygous 1/2 in 30.8% whereas 2/2 was not found, and its distribution was similar to the healthy controls. Likewise, IL-1B genotypes frequency in aggressive and chronic periodontitis: 1/1 – 75.0% and 65.6%; 1/2 – 25.0% and 33.4%; 2/2 – 0% and 0%, respectively did not alter markedly from the controls (59.7%, 36.5% and 3.9%, respectively). The aforementioned observations are in keeping with the findings of Papapanou et al. [14]. The authors demonstrated comparable distribution of IL-1A and IL-1B allele 2 in patients with periodontitis and healthy controls, 41.7% and 45.2%, respectively. Similarly, Sakellari et al. [20] reported no association of allele 2 of IL-1A and IL-1B genes and chronic periodontitis in a Greek population.

The present study does not support the notion of association between aggressive periodontitis and IL-1B polymorphism, and is in agreement with observations reported by Hodge et al. [21] as well as data from northern Europe, Hispanic population (central America) [22] and China [23]. Similarly to Diehl's et al. [19] report, a prevalence although not significant, of IL-1B 1/1 genotype in aggressive periodontitis patients (75.0%) in reference to healthy controls (59.7%) was found. Therefore, Diehl's et al. concluded that IL-1B polymorphism could be an important, but not a unique, determinant of periodontitis.

In the present study, no association between IL-1B polymorphism and periodontitis in non-smokers was revealed. Contrary to Kornman et al. [1], no significant differences in allelic and genotype distribution of IL-1B gene in non-smokers with periodontitis and all examined periodontal patients were observed. Similarly, Meisel et al. [24] did not find an influence of IL-1B genotype on periodontitis in non-smokers. However, the authors determined an association between complex genotype (allele 2 of IL-1A and IL-1B genes) and periodontitis in smokers.

As periodontitis is considered to be a disease to which many different factors may contribute, in the present study an interaction between IL-1B polymorphism, smoking habits and oral hygiene (API) was evaluated. Smoking contributes to periodontitis by affecting local circulation, immune system function and destruction of periodontal tissues [25]. Experimental data suggest that smoking impairs synthesis and secretion of IL-1B by macrophages, especially in subjects carrying allele 1 of IL-1B^{+3954}, and thus lead to promotion of periodontitis [26]. In the present study the multivariate regression analysis revealed significant association between smoking habits and API with periodontitis. No influence of IL-1B polymorphism was revealed. Smoking increased 10-fold the risk of periodontitis (OR=10.0, 95% CI 1.37-72.72, $p<0.05$), whereas API >50% more than 52-fold (OR=52.9, 95%CI 12.4-225.4, $p<0.05$). The association was seen in the case of aggressive periodontitis patients, where smoking increased more than 12-fold the disease risk (OR=12.4, 95%CI 1.3-121.9, $p<0.05$), and API >50% over 25-fold (OR=25.3, 95%CI 3.1-208.0, $p<0.05$). The multivariate analysis revealed significant association of API>50% with chronic periodontitis. The above observations support findings of other authors who reported an association between periodontitis and smoking as well as oral hygiene [27-30]. However, our results should be confirmed by other studies involving periodontitis patients recruited from a Polish population.

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Comparative research concerning clinical efficiency of three surgical methods of periodontium recessions treatment in five-year observations

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Abstract

Purpose: The aim of this study was a comparative analyses of clinical treatment efficiency of periodontium recessions after the application of double pedicle bilateral flap (DPBF), coronally repositioned flap in combination with connective tissue graft (CRF-CTG), coronally advanced flap in combination with guided tissue regeneration using collagen membranes (GTR-CM).

Material and methods: Research material consisted of 37 people (71.2% of initial patient number), including 27 women at the age from 17 to 53. All those people had single or multiple recessions, in I or II Miller's class, with the depth more than 2 mm. There were estimated 98 covered recessions of which 33 after DPBF, 41 after CRF-CTG and 24 after GTR-CM.

The clinical estimation of recession level before surgeries and after 12, 24, 60 months was done with the usage of the following parameters: recession depth (RD), recession width (RW), clinical attachment level (CAL) and keratinized tissue hight (HKT). There was also done an ultrasonic measurement of keratinized tissue thickness (TKT) in two groups of patients who had undergone surgeries CRF-CTG and GTR-CM. After 12, 24 and 60 months there were measured: an average percentage of a root coverage (%ARC), a percentage index of the complete root coverage (%CRC) and the percentage of complete coverage (CRC).

Results: Five-year inter group analyses of three surgical methods of recession treatment did not show any significant differences among surgeries for the following parameters: RD, CAL and TKT. The value of RD after DPBF was 0.85 mm, after CAF-CTG was 0.83 mm and after GTR-CM 0.38 mm.

There was a substantial difference of values such as ARC the best result of which was for the method GTR-CM (90%) and next for CRF-CTG (82%), CRC% and CRC with the best result for the methods GTR-CM (90%; 87.5%) and CRF-CTG (82.8%; 61%).

Conclusions: The authors' observations show that methods GTR-CM and CRF-CTG are mostly predictable and enable the stable coverage of periodotium recession during five-year observations.

Key words: periodontium recession, surgical treatment, five-year observations.

Introduction

One of the main assumptions of periodontium plastic surgery is to guarantee aesthetics of the red complex. This can be gained with the application of recession coverage and gingival augmentation [1,2]. The variety of surgical methods used during recession coverage enables to choose the most efficient method which in turns allows to gain the best therapeutic and aesthetic effect depending on the operated area condition [1-4]. Treatment with the usage of pedicle flaps allows to get a high percentage of average and complete recession coverage generally without an influence on width and thickness of keratinized gingiva, on condition that there is an appropriate amount of recipient tissue in the nearest recession area and an appropriate height of an oral cavity vestibule. Treatment with free gingival grafts results in no aesthetic healing effect described in science literature as the healing of scarf characteristics [1,2]. However, connective tissue grafts which can be made with the minimal amount of tissue in recipient place result in aesthetic rebuilt of the biological width and thickness of gingival and generally do not influence periodontium recession in the treated area. That is why there

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developed methods combining treatment applying pedicle flaps and connective tissue grafts with the alternative factors causing periodontium regeneration such as barrier membranes (GTR) and bioactive protein – enamel matrix protein and polypeptide growth factors [1,2]. The assessment of the recession coverage stability in a long-term evaluation should also be an important criteria to choices of treatment method [5].

The aim of this study was a five-year comparative assessment of periodontium recessions treatment efficiency after the application of: double pedicle bilateral flap acc. to Marggraf [6] (DPBF), coronally repositioned flap in combination with connective tissue graft acc. Bruno [7] (CRF-CTG), coronally advanced flap in combination with guided tissue regeneration with the usage of collagen membranes (GTR-CM) acc. Pini Prato [8] taking into consideration recommendations of Shieh and his co-operators [9].

Material and methods

37 people were assessed, at the age between 17 and 53 (the average age 30,31) including 27 women. Patients came for the medical check-up 5 years after the surgical treatment of periodontium recession coverage. It was 71.2% of the initial patient number. 98 covered recessions were tested 33 of which were after DPBF treatment, 41 after CRF-CTG and 24 after GTR-CM. The treatment was applied to 64 teeth in maxilla including 12 incisors, 33 canines and premolars, 34 teeth in mandibula including 10 incisors, 10 canines and 14 premolars. There were covered 38 singular recessions, 28 double recessions and a four-ply one.

The recessions qualified for the treatment were in I and II Miller's class, with height more than 2 mm occurring only on front teeth and premolars of maxilla and mandibula without fillings in the neck area which exceed the cemento-enamel junction. The initial keratinized gingival width of more than 3 mm and keratinized gingival thickness of more than 0.75 were recommendations for treatment DPBF or GTR-CM, whereas the smaller values for CRF-CTG.

The clinical assessment of recession progress before treatment and after 12, 24 and 60 months was done with the usage of the following parameters: recession depth (RD) and recession width (RW), clinical attachment level (CAL), keratinized tissue height (HKT, the distance measured between gingival margin and mucogingival junction). All measurement was done using periodontometer of Williams, scale calibrated at 1 mm. There was also done the ultrasonic mesured of keratinized tissue thickness (TKT) in a group of patients who had undergone treatment CRF-CTG and GTR-CM. The description of examination method was presented in a previous publication [10]. After 12, 24 and 60 months there were measured: average percentage of root coverage (%ARC), percentage index of complete root coverage (%CRC) and the percentage of complete root coverage (CRC).

During pre-surgical treatment the influence of potential aetiological factors of recession was lowered or eliminated. Special attention was paid to the appropriate technique of tooth cleaning and the proper hardness of a toothbrush. The correction of occlusal disturbances and premature contacts in centric occlusion and non-centric occlusion was done with a help of selective teeth grinding. Getting zero values of oral cavity hygiene was the condition of treatment application.

Before the treatment the surface of tooth roots was prepared by curette and diamonds of the lowest granularity.

A detailed description of treatment techniques was published earlier [11,12]. All surgical treatment was done by the same operating person whereas the clinical assessment by the different doctor.

During post-surgical treatment patients had avoided injuries of an operated place for 10 days. In that time they covered operated places with a paste Solcoseryl. Mouth rinsing with 0.12% chloredixine gluconate twice a day was ordered. After treatment GTR-CM it was ordered to use 1g of amoxicilinum once a day for 5 days which was in accordance with treatment protocols of other authors [8,13,14].

Statistical analyses

Average values, standard deviations and medians of all examined parameters were calculated for variables before treatment and after 12, 24 and 60 months. Verification of a hypotheses about equality of continual average parameters in particular groups was done using the t-Student test for couples. Verification of a hypothesis about equality of continual average parameters among treated groups was done with the usage of method of variance analyses (ANOVA) for groups of homogenous variance or with non-parametric Wilcox test (homogeneity was examined with Bartlett test). The level of significance $p \leq 0.05$ was assumed. During statistical analyses there was used a set of statistical computer programs EPINFO Ver. 3. 2, 2004.

Results

The average percentage of complete root coverage (%ARC), the value of a percentage index of complete recession coverage (%CRC) and the percentage of complete coverage in observations during 12, 24 and 60 months, in different treatment groups (in-group analyses) was illustrated in *Tab. 1-3* and comparison of values on inter-group analyses in the *Tab. 4*.

The highest average percentage of root coverage in 60-month observations was noticed for the method GTR-CM (90%, median 100%) next CRF-CTG (82,8%, median 100%) and DPBF (68.9%, median 75%). Consequently, the percentage index of complete recession coverage was 90% for GTR-CM, 82,8% for CRF-CTG and 71.2% for DPBF. Similarly, the percentage of complete coverage was the highest for the method GTR-CM (87.5%) next 61% for CRF-CTG and 45.5% for DPBF. During inter-group analyses for all mentioned parameters there existed significant differences among treatment in 60-month observation. Such a relation was not noticed after 12 and 24 months since treatment.

During in-group analyses for GTR-CM and CRF-CTG comparing the percentage values of an average and complete recession coverage with the percentage of complete coverage there were not noticed any crucial changes between yearly and two-year or five-year observation and between two-yar and five-year

Table 1. **Changes of average (ARC) and complete (CRC) percentage of recession coverage and the percentage index of complete recession treatment (%CRC) in 12- (1), 24- (2), 60- (5) month observation after the application of DPBF. Changes of recession width (RW) and depth (RD), clinical attachment level (CAL), keratinized tissue height (HKT), cemento-enamel junction to mucogingival junction distance (CEJ-MGJ) in pre-treatment observations (0), 12- (1), 24- (2), 60- (5) month observation after the application of DPBF**

	X	M	SD	0 vs 1	1 vs 2	2 vs 5	0 vs 2	0 vs 5	1 vs 5
ARC 1	86.7	100.0	24.2						
ARC 2	76.0	100.0	31.4		0.026*	0.208			0.011*
ARC-5	68.9	75.0	35.3						
%CRC1	85.7	100.0	24.9						
%CRC2	75.5	100.0	31.4		0.039*	0.363			0.031*
%CRC5	71.2	75.0	33.2						
CRC 1	0.697	1.000	0.467						
CRC 2	0.515	1.000	0.508		0.083	0.488			0.043*
CRC 5	0.455	0.000	0.506						
RD-0	2.88	3.00	0.78						
RD-1	0.394	0.000	0.659	0.00000*	0.019*	0.189	0.00000*	0.00000*	0.01*
RD-2	0.682	0.000	0.864						
RD-5	0.848	0.500	1.019						
RW-0	3.73	4.00	1.23						
RW-1	0.667	0.000	1.190	0.00000*	0.042*	0.255	0.00000*	0.00000*	0.010*
RW-2	1.35	0.00	1.55						
RW-5	1.61	1.25	1.71						
CAL-0	3.94	4.00	0.90						
CAL-1	1.33	1.00	0.65	0.00000*	0.0083*	0.296	0.00000*	0.00000*	0.0086
CAL-2	1.70	1.00	0.88						
CAL-5	1.88	2.00	1.02						
HKT-0	3.36	3.00	1.64						
HKT-1	3.88	4.00	2.01	0.101	0.182	0.182	0.389	0.802	0.036*
HKT-2	3.67	3.00	2.31						
HKT-5	3.45	3.00	2.37						
CEJ-MGJ-0	6.15	6.00	1.50						
CEJ-MGJ-1	4.30	4.00	1.83	0.00000*	0.564	0.444	0.00001*	0.00001*	0.821
CEJ-MGJ-2	4.41	4.00	2.26						
CEJ-MGJ-5	4.24	4.00	2.28						

DPBF – double pedicle bilateral flap; x – average; SD – standard deviation; M – median; * – statistical significance

observations. However, there appeared significant differences among yearly and five-year results for DPBF. Such a relation was not noticed between yearly and two-year observations and between two-year and five-year observations.

After the treatment with separate treatment methods, changes of recession depth and width, clinical attachment level, width and thickness of keratinized gingiva and the distance between cemento-enamel junction and mucogingival junction were illustrated in *Tab. 1-3*. The initial recession depth was significantly different in separate treatment groups. The deepest recessions were noticed in a group CRF-CTG (4.54 mm), and the lowest in a group DPBF (2.88 mm). During 5-year observation there appeared a big reduction of RD and RW in all treatment groups. However, there were no significant changes between yearly and two-year or five-year observations and between two-year and five-year observations for GTR-CM and CRF-CTG. Nevertheless, it should be emphasized that 5 years after the recession coverage treatment with the method of guided tissue regeneration with the usage of collagen membranes there was noticed a small decrease of an average recession depth comparing with two-year observation (by 0.21 mm). It was still the value not significantly higher then the one noticed during 12-month observations. The similar relation concerned recession width which average value decreased by 0.46 mm. However, an application of method DPBF resulted in a big increase of recession depth and width between 12, 24 and 60 months, still this value was significantly smaller in comparison to results before treatment. There were no big differences of recession depth and width between 2-year and 5-year observations. In inter-group analyses, 12 and 24 months after the treatment, there were no big changes of recession depth and width among examined groups (*Tab. 4*). 60 months after the treatment there were no big changes of RD but the significant one of RW. The smallest recession width was in GTR-CM group (0.54) and the biggest in DPBF (1.61).

There appeared significant changes in the position of clinical attachment level among examined groups before the treatment. The initial average loss of CAL was the biggest in a group of patients for which there was planned CRF-CTG (5.59 mm) and next GTR-CM (5.00 mm). The rebuilt of the attachment was crucial for all examined groups during 12-, 24- and 60-month observations. In addition, there appeared a significant increase of CAL after 24 and 60 months in comparison to initial

***Table 2*. Changes of average (ARC) and complete (CRC) percentage of recession coverage and the percentage index of complete recession treatment (%CRC) in 12- (1), 24- (2), 60- (5) month observation after the application of CRF-CTG. Changes of recession width (RW) and depth (RD), clinical attachment level (CAL), keratinized tissue height (HKT), cemento-enamel junction to mucogingival junction distance (CEJ-MGJ) in pre-treatment observations (0), 12- (1), 24- (2), 60- (5) month observations after the application of CAF-CTG. Changes of keratinized tissue thickness (TKT) in pre-treatment observation (0) and 12- (1), 24- (2) and 60- (5) month observations after the application of CAF-CTG**

	X	M	SD	0 vs 1	1 vs 2	2 vs 5	0 vs 2	0 vs 5	1 vs 5
ARC 1	88.8	100.0	20.7						
ARC 2	85.3	100.0	20.2		0.207	0.340			0.101
ARC-5	82.8	100.0	24.0						
%CRC1	86.4	100.0	24.9						
%CRC2	85.5	100.0	20.2		0.810	0.312			0.423
%CRC5	82.8	100.0	24.0						
CRC 1	0.732	1.000	0.449						
CRC 2	0.610	1.000	0.494		0.096	1.00			0.133
CRC 5	0.610	1.000	0.494						
RD-0	4.54	4.00	1.50						
RD-1	0.610	0.000	1.070	0.00000*	0.473	0.421	0.00000*	0.00000*	0.277
RD-2	0.732	0.000	1.073						
RD-5	0.829	0.000	1.202						
RW-0	4.32	4.00	1.06						
RW-1	1.02	0.00	1.78	0.00000*	0.570	0.897	0.00000*	0.00000*	0.555
RW-2	1.15	0.00	1.75						
RW-5	1.17	0.00	1.66						
CAL-0	5.59	5.00	1.47						
CAL-1	1.49	1.00	1.03	0.00000*	0.048*	0.098	0.00000*	0.00000*	0.006*
CAL-2	1.77	1.00	1.10						
CAL-5	2.02	2.00	1.23						
HKT-0	1.32	1.00	1.25						
HKT-1	4.61	4.00	1.28	0.00000*	0.920	0.921	0.00000*	0.00000*	0.832
HKT-2	4.63	5.00	1.61						
HKT-5	4.66	5.00	1.33						
CEJ-MGJ-0	5.90	6.00	1.69						
CEJ-MGJ-1	5.12	5.00	1.68	0.009*	0.478	0.417	0.05*	0.248	0.136
CEJ-MGJ-2	5.29	5.00	1.83						
CEJ-MGJ-5	5.51	5.00	1.45						
TKT-0	0.649	0.640	0.166						
TKT-1	1.48	1.26	0.51	0.00000*	0.014*	0.0201*	0.00000*	0.00000*	0.0007*
TKT-2	1.26	1.35	0.37						
TKT-5	1.17	1.20	0.32						

CAF-CTG – coronally repositioned flap in combination with connective tissue graft; x – average; SD – standard deviation; M – median; * – statistical significance

condition and a year after the treatment, all this for DPBF and CRF-CTG. There was no big increase between 2-year and 5-year observations. However, the method GTR-CM did not result in any significant changes of the position of clinical attachment level between yearly and 2-year observations and between 2-year and 5-year ones. During in-group analyses there were no big differences among groups in all examined periods.

The average distance between cemento-enamel junction and mucogingival junction was the biggest in a group treated with GTR-CM (7.17 mm) and the smallest in CRF-CTG (5.90 mm). There was no significant change of this parameter in comparison of three treatment methods before the treatment. For DPBF and GTR-CM the distance increased significantly during 12, 24 and 60 months after the treatment compared to the initial state. For CRF-CTG method the relation in 12- and 24-month observations was similar, and after 5 years the average value of that parameter did not differ much from before-treatment state. In inter-group analyses there were big differences among treatment during 5-year observations. The biggest noticed value was for GTR-CM, the smallest for DPBF.

The average initial width of keratinized gingiva was significantly different in examined treatment groups what was the consequence of pre-treatment qualifications. The smallest value was in recession group treated with CRF-CTG (1.32 mm). For methods DPBF and GTR-CM those values were quite similar (3.36 mm and 3.38 mm). Taking into consideration the comparison of results of pre-treatment results and those after 12, 24 and 60 months it should be emphasized that only methods CRF-CTG and GTR-CM resulted in big increase of HKT one year after the treatment. This relation stayed the same during two years after the treatment only for CRF-CTG method. The significant increase of keratinized gingiva width was noticed again for CRF-

Table 3. **Changes of average (ARC) and complete (CRC) percentage of recession coverage and the percentage index of complete recession treatment (%CRC) in 12- (1), 24- (2), 60- (5) month observation after the application of GTR-CM.. Changes of recession width (RW) and depth (RD), clinical attachment level (CAL), keratinized tissue height (HKT), cemento-enamel junction to mucogingival junction distance (CEJ-MGJ) in pre-treatment observations (0), 12- (1), 24- (2), 60- (5) month observations after the application of GTR-CM. Changes of keratinized tissue thickness (TKT) in pre-treatment observation (0) and 12- (1), 24- (2) and 60- (5) month observations after the application of GTR-CM**

	X	M	SD	0 vs 1	1 vs 2	2 vs 5	0 vs 2	0 vs 5	1 vs 5
ARC 1	91.3	100.0	23.2						
ARC 2	85.8	100.0	27.5		0.314	0.341			0.769
ARC5	90.0	100.0	28.9						
%CRC1	95.0	100.0	14.4						
%CRC2	85.8	100.0	27.5		0.108	0.341			0.441
%CRC5	90.0	100.0	28.9						
CRC 1	0.792	1.000	0.415						
CRC 2	0.750	1.000	0.442		0.664	0.082			0.328
CRC 5	0.875	1.000	0.338						
RD-0	3.79	4.00	1.41						
RD-1	0.250	0.000	0.737	0.00000*	0.201	0.170	0.00000*	0.00000*	0.632
RD-2	0.583	0.000	1.213						
RD-5	0.375	0.000	1.135						
RW-0	4.38	4.50	1.38						
RW-1	0.750	0.000	1.567	0.00000*	0.398	0.126	0.00000*	0.00000*	0.512
RW-2	1.00	0.00	1.82						
RW-5	0.542	0.000	1.474						
CAL-0	5.00	5.00	1.32						
CAL-1	1.33	1.00	0.82	0.00000*	0.072	0.082	0.00000*	0.00000*	0.616
CAL-2	1.67	1.00	1.20						
CAL-5	1.42	1.00	1.08						
HKT-0	3.38	3.50	2.04						
HKT-1	4.25	4.00	1.07	0.020*	0.089	0.111	0.110	0.040*	0.777
HKT-2	3.96	4.00	1.12						
HKT-5	4.31	4.50	0.93						
CEJ-MGJ-0	7.17	7.50	2.65						
CEJ-MGJ-1	4.58	4.00	1.50	0.00007*	0.426	0.339	0.00000*	0.0003*	0.654
CEJ-MGJ-2	4.42	4.50	1.56						
CEJ-MGJ-5	4.69	5.00	1.35						
TKT-0	0.743	0.750	0.110						
TKT-1	1.27	1.20	0.31	0.00000*	0.264	0.00006*	0.00000*	0.00000*	0.007*
TKT-2	1.18	1.14	0.34						
TKT-5	1.05	1.04	0.25						

GTR-CM – guided tissue regeneration using collagen membranes, x – average, SD – standard deviation, M – median, * – statistical significance

CTG and GTR-CM in 5-year observation. 24 months after the treatment there was no crucial change of HKT in a recession group covered with guided tissue regeneration using collagen membranes. However, the next three years showed the average (not significant) growth of gingival width by 0.35 mm 60 months after the treatment and usage of the method DPBF that parameter did not changed much in comparison with an initial state and after 12 and 60 months HKT got much smaller. Application of CRF-CTG and GTR-CM, comparing results after 12 months with 24 months and 24 with 60 months, did not change significantly the value of that parameter. Big changes of keratinized gingiva width were noticed after 24 and 60 months in inter-group analyses. The highest values were noticed for the method CRF-CTG (4.63 mm and 4.66 mm). The smallest values (and continually getting smaller) for DPBF (3.67 mm and 3.45 mm).

The initial average thickness of keratinized gingival of the recessions treated with CRF-CTG and GTR-CM was much smaller in patients qualified for treatment CRF-CTG (0.65 mm) which resulted from conditions of qualifications before treatment. There was noticed a small decrease of keratinized gingiva thickness in comparison between examination results before treatment and after 12, 24 and 60 months. In addition, 5 years after the treatment with both methods the average thickness increase still remained compared with the initial state, bigger for CRF-CTG (1,17 mm) than for GTR-CM (1.05 mm). Usage of connective tissue graft method resulted in the significant decrease of that value compared with observations between 12, 24 and 60 months and between 24 and 60 months. For GTR-CM the situation was stable between 12 and 24 months, next the value of that parameter decreased significantly. There were no big changes of average TKT values in inter-group analyses during 12, 24 and 60 months (*Tab. 4*).

***Table 4*. Inter-group analysis of average (ARC) and complete (%CRC) percentage of recession coverage and the percentage of complete recession treatment (CRC), recession width (RW) and depth (RD), clinical attachment level (CAL), keratinized tissue height (HKT), cemento-enamel junction to mucogingival junction distance (CEJ-MGJ) in pre-treatment observations (0), 12- (1), 24- (2), 60- (5) month observations. Changes of keratinized tissue thickness (TKT) in pre-treatment observation (0) and 12- (1), 24- (2) and 60- (5) month observations after the application of DPBF, CAF-CTG and GTR-CM**

	DPBF			CRF-CTG			GTR-CM			P
	X	M	SD	X	M	SD	X	M	SD	
ARC 1	86.7	100.0	24.2	88.8	100.0	20.7	91.3	100.0	23.2	0.744
ARC 2	76.0	100.0	31.4	85.3	100.0	20.2	85.8	100.0	27.5	0.240
ARC-5	68.9	75.0	35.3	82.8	100.0	24.0	90.0	100.0	28.9	0.01*
%CRC1	85.7	100.0	24.9	86.4	100.0	24.9	95.0	100.0	14.4	0.239
%CRC2	75.5	100.0	31.4	85.5	100.0	20.2	85.8	100.0	27.5	0.199
%CRC5	71.2	75.0	33.2	82.8	100.0	24.0	90.0	100.0	28.9	0.044*
CRC 1	0.697	1.000	0.467	0.732	1.000	0.449	0.792	1.000	0.415	0.732
CRC 2	0.515	1.000	0.508	0.610	1.000	0.494	0.750	1.000	0.442	0.202
CRC 5	0.455	0.000	0.506	0.610	1.000	0.494	0.875	1.000	0.338	0.005*
RD-0	2.88	3.00	0.78	4.54	4.00	1.50	3.79	4.00	1.41	0.000*
RD-1	0.394	0.000	0.659	0.610	0.000	1.070	0.250	0.000	0.737	0.274
RD-2	0.682	0.000	0.864	0.732	0.000	1.073	0.583	0.000	1.213	0.859
RD-5	0.848	0.500	1.019	0.829	0.000	1.202	0.375	0.000	1.135	0.222
RW-0	3.73	4.00	1.23	4.32	4.00	1.06	4.38	4.50	1.38	0.0627
RW-1	0.667	0.000	1.190	1.02	0.00	1.78	0.750	0.000	1.567	0.829
RW-2	1.35	0.00	1.55	1.15	0.00	1.75	1.00	0.00	1.82	0.740
RW-5	1.61	1.25	1.71	1.17	0.00	1.66	0.542	0.000	1.474	0.025*
CAL-0	3.94	4.00	0.90	5.59	5.00	1.47	5.00	5.00	1.32	0.000*
CAL-1	1.33	1.00	0.65	1.49	1.00	1.03	1.33	1.00	0.82	0.684
CAL-2	1.70	1.00	0.88	1.77	1.00	1.10	1.67	1.00	1.20	0.922
CAL-5	1.88	2.00	1.02	2.02	2.00	1.23	1.42	1.00	1.08	0.112
HKT-0	3.36	3.00	1.64	1.32	1.00	1.25	3.38	3.50	2.04	0.000*
HKT-1	3.88	4.00	2.01	4.61	4.00	1.28	4.25	4.00	1.07	0.091
HKT-2	3.67	3.00	2.31	4.63	5.00	1.61	3.96	4.00	1.12	0.018*
HKT-5	3.45	3.00	2.37	4.66	5.00	1.33	4.31	4.50	0.93	0.001*
CEJ-MGJ-0	6.15	6.00	1.50	5.90	6.00	1.69	7.17	7.50	2.65	0.100
CEJ-MGJ-1	4.30	4.00	1.83	5.12	5.00	1.68	4.58	4.00	1.50	0.112
CEJ-MGJ-2	4.41	4.00	2.26	5.29	5.00	1.83	4.42	4.50	1.56	0.088
CEJ-MGJ-5	4.24	4.00	2.28	5.51	5.00	1.45	4.69	5.00	1.35	0.006*
TKT-0				0.649	0.640	0.166	0.743	0.750	0.110	0.018*
TKT-1				1.48	1.26	0.51	1.27	1.20	0.31	0.077
TKT-2				1.26	1.35	0.37	1.18	1.14	0.34	0.344
TKT-5				1.17	1.20	0.32	1.05	1.04	0.25	0.135

DPBF – double pedicle bilatreral flap; CAF-CTG – coronally repositioned flap in combination with connective tissue graft; GTR-CM – guided tissue regeneration using collagen membranes; x – average; SD – standard deviation; * – statistically significance

Discussion

Authors' observations show that methods GTR-CM and CRF-CTG are the most predictable and guarantee the stable recession coverage in 5-year observation. All parameters describing recessions and mucogingival parameters in a group treated with guided tissue regeneration with the usage of collagen membranes improved one year after the treatment keeping such an important level for 5 years. The only exception is keratinized gingiva thickness which after 2-year stable increase got significantly lower but still by the value much higher in comparison with the initial state. Although keratinized gingival width decreased a little two years after the treatment it increased during next three years reaching the value similar to the one noticed one year after the treatment. The average distance CEJ-MGJ observed in time is significantly different in comparison with the initial state (decrease by 2.48 mm in 5-year observation) but during all that time it kept increasing slightly. On the one hand, this process may be caused by genetic determination of mucogingival junction location [15], on the other hand by the existence of an increase of keratinized gingival width in that time. The obtained increase of HKT in 24-60-month observation (0.35 mm) and the increase of CEJ-MGJ by 0.27 in the same time are close which in that case can point at complicated

aetiology of that process. In addition the increase of keratinized gingival width may also result from the phenomena of creeping attachment (CA). During 24-60-month observation there was CA of an average value of 0.21 mm for recessions treated with GTR-CM. The decrease of CAL in the same time may also be the result of that process. However, tissue regeneration process is responsible for rebuilt of CAL in GTR-CM method. From the histological point of view it is observed that there exists the rebuilt of root cement with the set connective tissue fibres and limited regeneration of cortical lamina of alveolar process [16-18]. Such support of soft tissues can improve the stability of a therapeutic effect even in a long-term observations [19].

Examination results for CRF-CTG method show similar values. During 60 months the following values were maintained: the significant decrease of recession depth and width, the significant increase of keratinized gingiva width and thickness and the rebuilt connective tissue attachment level. Only the value of CEJ-MGJ was close to the initial state after 60 monts.The second parameter which changed during 5-year observations was clinical attachment level. The second year after treatment was a critical moment at the end of which the significant decrease of CAL appeared. Next this value maintained on a little lower level until 60 months after the treatment. During that time (between 12, 24 and 60 months, between 24 and 60 months) TKT decreased much, however, reaching twice bigger value compared with the initial one (1.17 mm). In addition, the increase of keratinized gingival width tripled (4.61 mm) during the first year and remained on that level throughout a year of study. It seems that this process is caused by grafted tissue from palate chewing area which by keeping the ability to induce epithelial cells of a covering flap to keratinization, decides on vastness of keratinized gingival rebuilt [20]. That is why it is possible to reach the increase of HKT in a yearly observation and to keep it stable due to the rebuilt of mucogingival complex. However, the way of tissue healing can be responsible for changes of CAL for CRF-CTG method. The results of histological research in this case are controversial. Some authors claim that only tissue reparation is possible [21] whereas the others talk about partial regeneration [22,23].

But the usage of DPBF, a year after the treatment, resulted in significant improvement of all parameters except for HKT. Keratinized gingival width increased a little by 0.52 mm and during 60 months slowly decreased reaching the averaged value only 0.09 mm bigger than the initial one. The comparison of examination results during 12-60-month observation shows the significant changes of all parameters except for CEJ-MGJ. This value (0.17 mm) decreased a little between 24 and 60 months what can be responsible for the decrease of HKT (0.22 mm) in that time. It seems to be crucial that during 24-60-month observation all analyzed results changed only a little which can prove the stability of therapeutic effect obtained two years after the treatment.

Doing the inter-group analyses of three surgical methods of recession treatment it should be noted that there were no significant differences among surgical treatment concerning only recession height, clinical attachment level and keratinized gingiva thickness. The significant differences apply to average recession coverage with the best result for GTR-CM method and next CRF-CTG, the value of the percentage index of complete recession treatment and the percentage of complete coverage, also with the best results for GTR-CM and CRF-CTG. It is possible that such results are due to smaller number of patients that attended controlling examinations compared with the initial group. After treatment with DPBF the number of assessed recessions decreased by 12, after CRF-CTG it decreased by 13 and after GTR-CM there were assessed only 5 recessions. In addition, in CRF-CTG group 3 persons with recognized progressive recession (Miller II class) in long-term observations have undergone orthodontic treatment due to occlusion abnormalities. It is difficult to say whether this factor could be responsible for the significant decrease of recession depth in those patients. Nevertheless only long-term assessment after orthodontic treatment can confirm or exclude this aetiological factor. Soon after the treatment, in one patient of this group there appeared disordered in-healing of connective tissue graft. The early age of the patient and lack of tooth abnormalities which could be responsible for occlusion abnormalities forced the detailed analyses of this case. The additional diagnostic examinations proved the existence of ascending character of movement system, because of changes in muscle tension in biocinematic chain, led to incorrect relation between maxilla and mandibula (flat occlusion plane) and consequently to overload responsible for the development of multiply gingival recessions. The detailed description of that case was presented in different research publication [24]. As we can see there are many factors deciding on the success of a given treatment method, including an effective elimination of modifying aetiological factors, keeping to qualification conditions before treatment characteristic for a given operating technique [25], treatment procedure (both tools, used material and the way of treatment application) [26,27], operating doctor experience and patient monitoring during the longest possible time after the treatment [3,4,28-29].

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Autogenous bone and platelet-rich plasma (PRP) in the treatment of intrabony defects

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Abstract

Purpose:

– obtaining an answer to the question whether autogenous bone in combination with PRP give a therapeutical effect in the form of periodontal ligament attachment regeneration,

– defining the degree of elimination of a convenient environment for subgingival bacterial plaque by reduction of periodontal pocket depth and periodontitis.

Material and methods: Twenty-six systematically healthy patients with diagnosed chronic and advanced periodontitis (24 females and 2 males) were selected for the study. In general 72 periodontal infrabony pockets were treated.

Clinically the following indexes were examined and measured:

1. Plaque Index by Silness and Löe
2. Sulcus Bleeding Index by Mühlemann and Son
3. Clinical Attachment Level (mm)
4. Pocket Depth (mm)
5. Gingival Recession (mm)
6. Tooth mobility with the use of Periotest
7. Degree of alveolar bone loss with the use of Engelberger, Marthaler and Rateitschak index – EMR Index.

Results: At 12 months after treatment the following results were noted:

– mean value of attachment level regeneration 3.47 mm
– mean value of pocket depth decreased by 3.7 mm
– mean value of tooth mobility reduction by 48.3%
– regeneration of alveolar bone by 9.24%.

Conclusions:

1. Autogenous bone with added PRP in treatment of intrabony defects caused by periodontitis have given significant clinical improvement of the periodontal tissues.

2. The combination of PRP and autogenous bone caused the elimination of a convenient environment for subgingival bacterial plaque eliminating periodontitis.

Key words: Widman's procedure, bone regeneration, autograft bone, platelet-rich plasma.

Introduction

Existing inflammation of the periodontium leads to destruction of periodontal tissues often causing a loss of some or even all teeth, whereas untreated vertical bone defects lead to a serious function impairment of the stomatognathic system. An important supplement of general complex periodontal treatment is periodontal surgery. Thanks to these periodontal surgical procedures there is a chance for restoring tooth supporting tissues. Up-to-date surgical treatment methods allow to avoid unwanted teeth extraction and widen possibilities of stomatognathic system rehabilitation.

The study aim was to:

– obtain an answer to the question whether cancellous cortical bone in combination with PRP give a therapeutic effect in the form of periodontal ligament regeneration,
– define the degree of elimination of a convenient environment for subgingival bacterial plaque by reduction of periodontal pocket depth and periodontitis.

Material and methods

Twenty-six systematically healthy patients with diagnosed chronic and advanced periodontitis (24 females and 2 males)

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Table 1. **Mean values of chosen clinical parameters at different periods of observation after implantation of autogenous bone with added PRP**

Measured parameters	Before surgery	3 months after surgery	6 months after surgery	12 months after surgery
Number of treated periodontal pockets	72	72	72	72
PI	0.60	0.47	0.38	0.32
SBI	1.36	0.76	0.36	0.09
CAL	8.41	5.34 (+36.8%)	5.29 (+37.3%)	4.94 (+40.8%)
PD	6.62	3.28 (+50.0%)	3.18 (+51.4%)	2.92 (+55.2%)
REC	2.01	2.32 (+26.0%)	2.26 (+23.3%)	2.14 (+18.7%)
Tooth mobility	20.03	15.76 (24.5%)	13.01 (36.8%)	10.35 (48.3%)
EMR	41.15%	n.d.	n.d.	50.39%

n.d. – no data

were selected for the study, mean age 46,8 years, range from 21 to 62 years.

Patients were selected basing on clinical periodontal examination and panoramic radiogram analysis. Exclusion criteria consisted of patients with typical horizontal alveolar bone defects. Inclusion criteria consisted of patients having vertical intrabony defects. Generally 72 periodontal infrabony pockets were treated, 53 by upper teeth (25 by incisors, 5 by canines, 10 by premolars, 13 by molars) and 19 periodontal pockets localized in the mandible (1 by a canine, 8 by premolars, 10 by molars). The examined group consisted of twenty-one 3-wall defects, fifty 2-wall defects and one 1-wall defect.

Clinically the following indexes were examined and measured:

1. Plaque Index (PI) of Silness and Löe [1]
2. Sulcus Bleeding Index (SBI) of Mühlemann and Son [2]
3. Clinical Attachment Level – CAL (mm)
4. Pocket Depth – PD (mm)
5. Gingival Recession – REC (mm)
6. Tooth mobility with the use of Periotest
7. Degree of alveolar bone loss with the use of Engelbelger, Marthaler and Rateitschak Index – EMR Index [3,4]

Patients were informed as to the character and aim of the study and signed an informed consent. Initial therapy consisted of oral hygiene instructions, tooth brushing using the roll method and cleaning of interdental spaces using a system individually chosen for the patients' needs. Scaling and root planning was performed. Occlusal adjustment of fillings and prostodonthic restorations was carried out, also elimination of iatrogenic irritative factors, carries was treated, endodontical treatment was carried out, occlusal analysis and where necessary occlusal adjustment was performed.

Four to six weeks following the first hygienic phase a control hygiene examination was carried out among the patients using the Plaque Index. Only patients with good oral hygiene (PI: 0.4--0.6) were qualified for surgical treatment [5].

Directly before surgery 8.5 ml of blood were drawn from the antecubital vein from each patient to a glass tube containing a CPDA solution as an anticoagulant. A special glass tube set PRP-kit (Curasan Pharma) and laboratory centrifuge MPW – 221/MPW – 223 (Curasan Pharma) were used to obtain separation of basic blood fractions (PRP) from the patients blood.

Osseous tissue was taken directly from the surgical site with the use of Safescraper (C.G.M.s.p.a. Divisione Medicale Meta).

In all patients with diagnosed intrabony defects the modified flap Widmans procedure was carried out and autogenous bone was implanted with added platelet-rich plasma (PRP).

Results

The results showed that the treated patients maintained good oral hygiene. Mean value of Plaque Index (PI) achieved before treatment was 0.6 and at 12 months was reduced to 0.3. It was observed that the Sulcus Bleeding Index (SBI) was reduced after 12 months to a value of 0.1 (*Tab. 1*).

Comparing mean values of clinical attachment level, a significant reduction was observed in all study periods, giving evidence for its regeneration (*Fig. 1*). At 6 months postoperatively clinical attachment gain was observed of mean value 3.1 mm and at 12 months postoperatively this value reached 3.5 mm.

One of the desired outcomes of periodontal regenerative procedures is pocket depth reduction. In the study after a period of 6 months the mean pocket depth value was 3.2 mm, after 12 months a reduction by 2.9 mm was observed (*Fig. 2*). The total value of pocket depth reduction after a year was 3.7 mm. This

Figure 1. **Mean values of changes in attachment level CAL (in mm) at determined observation periods**

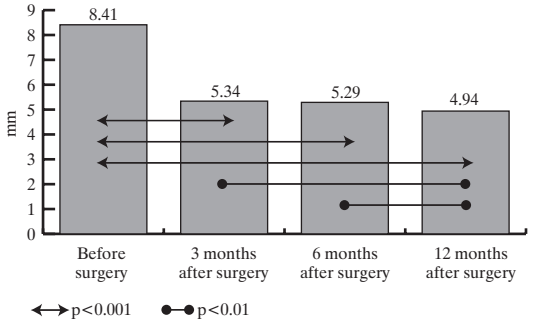


Figure 2. **Mean value of periodontal pocket depth PD (in mm) at determined observation periods**

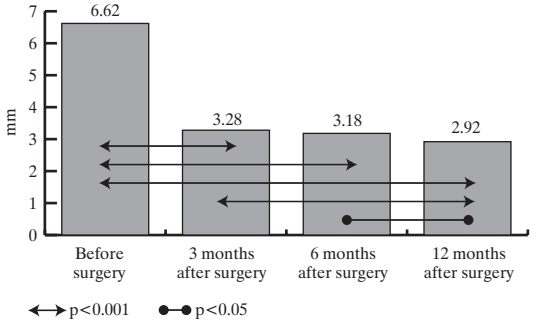


Figure 3. **Values of the radiological alveolar bone defect index EMR in percentage**

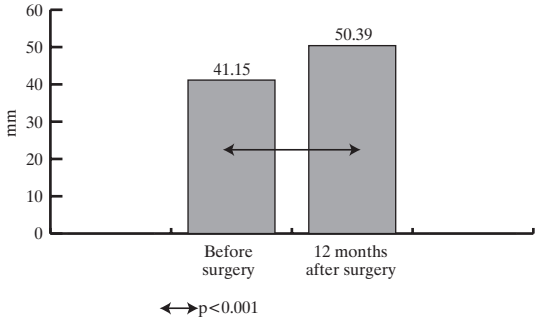
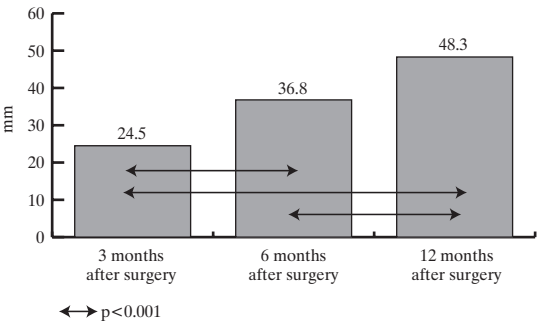


Figure 4. **Reduction of tooth mobility at different periods of observation compared to baseline (in percentage)**



shows that a combination of autogenous bone implantation and PRP is a highly effective treatment.

Carried out analysis of gingival recession showed an increase by 0.13 mm after 12 months postoperatively in comparison to baseline (*Tab. 1*).

To define the degree of bone defect progression the EMR index was used. According to this index the correct value for alveolar bone level both in the mandible and maxilla is in the range of 60.0% to 70.0% [3,4]. The EMR value before treatment was 41.15%. At 12 months postoperatively the EMR index presented the value of 50.4% (*Fig. 3*). The obtained results show that significant alveolar bone regeneration was achieved of 9.2%.

An important outcome of surgical periodontal treatment is tooth mobility reduction, what gives a great chance for tooth maintenance or even its incorporation in prosthetic treatment. The study results have confirmed that treatment of periodontal infrabony pockets with the use of autogenous bone and PRP caused reduction of tooth mobility by a value of 48.3% (*Fig. 4*).

Discussion

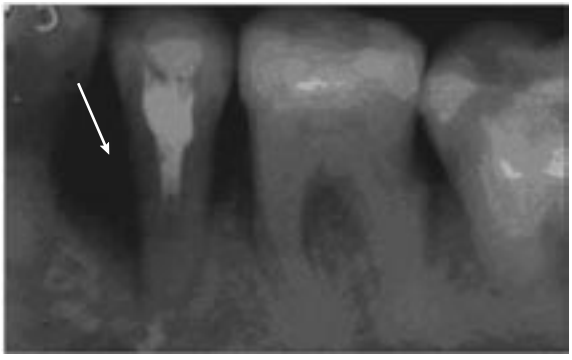
Process complexity undergoing during periodontal regeneration is the cause for utilizing a variety of biomaterials, which induce mineralized tissues (cementum and alveolar bone) and soft tissues (periodontal ligament, periodontium) formation. The aim of regenerative periodontal procedures is to achieve regeneration of all periodontal tissues, that is tissues, which are functionally and structurally identical with those lost as a result of a disease process [6,7]. Unfortunately it is impossible to supply documentary evidence for periodontal regeneration in clinical conditions. It also has to be emphasized that reduction of periodontal pocket depth after carried out treatment does not allow to clearly ascertain that a new periodontal ligament has been formed. In some cases clinical measurements are not consistent with histological findings [8] and clinical attachment gain does not always imply regeneration of the periodontal ligament at the histological level [9].

The most biocompatible implant material used in treatment of intrabony bone defects caused by periodotitis is autogenous bone. Carraro at al. [10] performed treatment of intrabony defects using autogenous bone and 12 months postoperatively recorded regeneration of the periodontal ligament by 2.88 mm. In a study by Froum at al. [11] treatment with autogenous bone was followed by re-entry surgery and presented regeneration of alveolar bone by 2.98 mm while results of open curettage showed bone regeneration of only 0.66 mm. A more intensive bone formation, of 3.4 mm after implantation of autogenous bone, was observed by Hiatt at al. [12].

Recently developed procedures for treatment of intrabony defects utilize platelet-rich plasma (PRP), a concentrated suspension of growth factors. Various studies have preclinically examined the use of growth factors on animals [13-18]. Local application of growth factors is used to promote healing, especially periodontal regeneration. Many studies have shown that PDGF, IGF, TGF-β are found in PRP and the use of these factors has led to promising results also in humans [19-21].

Figure 5. **A radiogram showing a bone defect around tooth 35 treated with autogenous bone and PRP**

a) before treatment



b) 12 months after treatment

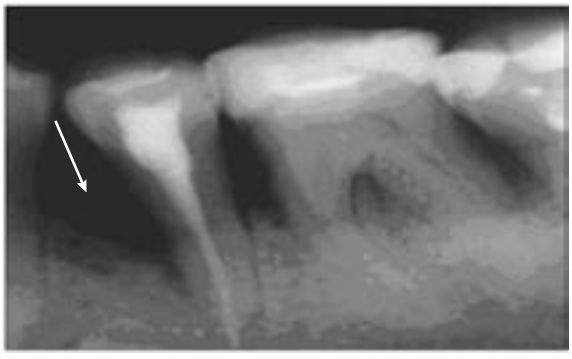
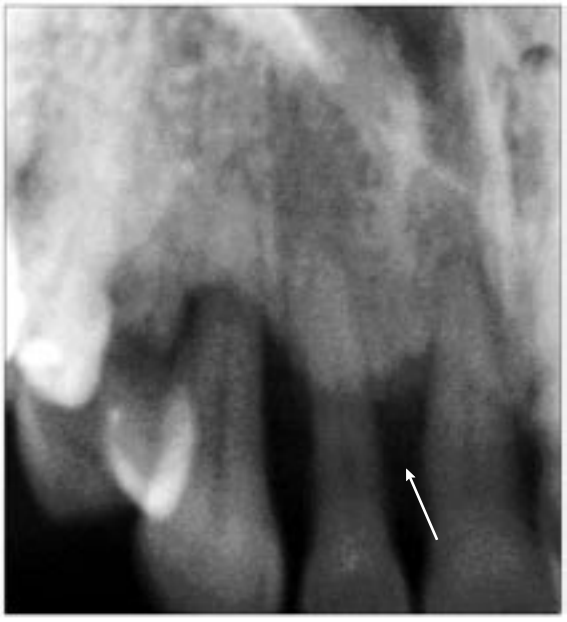
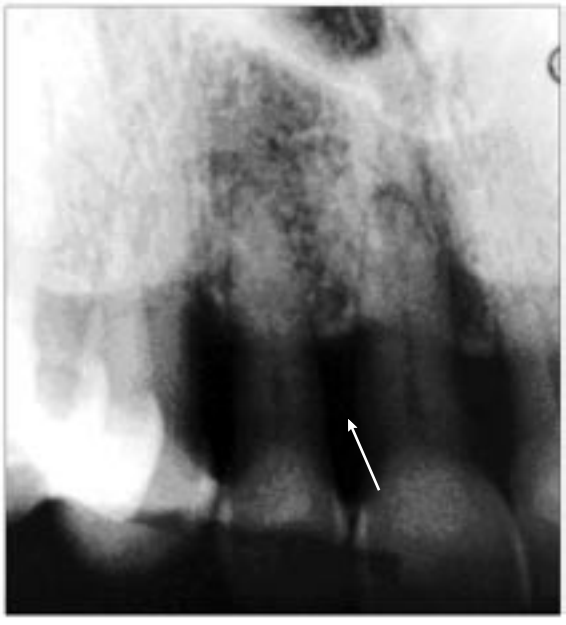


Figure 6. **A radiogram showing a bone defect around tooth 12 treated with autogenous bone and PRP**

a) before treatment



b) 12 months after treatment



A combination of these growth factors with biomaterials can stimulate regeneration of bone [19-21]. Bone is constantly remodeled by cycles of resorption and formation of its structure and both cycles of this remodeling are controlled by locally released growth factors [22].

Very few studies have been carried out with the purpose to estimate the efficiency of use of combination of PRP and autogenous bone on bone regeneration. Studies by Marx at al. have given evidence that the addition of PRP to milled bone graft obtained from the posterior illium increased the rate of bone formation and the final quantity of bone formed at 6 months postoperatively [21]. These authors have shown that bone grafts with added PRP presented with increased bone density (74.0%+/-11.0%) in comparison with grafts without PRP (55.1% +/- 8.0%). Results of the above mentioned studies suggested that growth factors accelerate and intensify alveolar bone regeneration. Similar conclusions have been published by Fennis at al. [23] in a study carried out on bone regeneration in a goat.

In opposition to these findings stands Aghaloo at al. [24]. Basing on carried out studies these authors suggest that there is no evident relevance between PRP additions to bone grafts and increased bone regeneration. The authors based this evaluation on a study of regeneration process effectiveness on rabbits cranial vault. Similar observations were published by Jakse at al. [25] in an experimental study on autogenous sinus grafts in sheep.

Results of the present study confirm data obtained by Aghaloo at al. and give evidence of not significant effectiveness of autogenous bone with PRP in treatment of intrabony defects resulting from periodontitis. Results of the present study indicate alveolar bone regeneration in the range of 10.0% (9.24%), while more effective results have been obtained with guided tissue regeneration of periodontal tissues with the use of membranes, regeneration methods and biomaterials [9,19,20].

Autogenous bone and PRP are autologous preparations without antygenicity, this allows to eliminate biological concerns such as immunogenic reactions and disease transmission. Moreover, autologous bone cells are vital, contain receptors of growth factors PDGF and TGF-β [21] and also show osteogenetic and osteoinduction action.

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The concentration of anthranilic acid in saliva of orthodontic appliances

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Abstract

Purpose: **Anthranilic acid is an important, the aromatic intermediate in the degradation of tryptophan in kynurenine pathway. This compound plays an important role in the regulation of immunological processes as well shows antibacterial activity. The aim of our study was to estimate the concentration of anthranilic acid in saliva of young patients with orthodontic apparatus. We also assessed correlation between saliva anthranilic acid concentrations and time of orthodontic treatment. For the first time we have demonstrated the enhanced concentration of anthranilic acid in saliva of young orthodontic appliances.**

Material and methods: **The study was performed on non-stimulated, mixed saliva of patients with orthodontic appliances. The concentration of anthranilic acid and was determined by high-performance liquid chromatography (HPLC).**

Results: **The concentration of anthranilic acid was significantly higher in orthodontic patients ($p=0.043$) in comparison to healthy volunteers. The mean time of orthodontic treatment was 15.0 ± 2.03 months. We did not observe existence of correlation between anthranilic acid concentration in saliva and time of orthodontic treatment ($r=-0.250$; $p=0.517$).**

Conclusion: **These results might indicate that anthranilic acid can be one of many factors initiating of periodontal disease in orthodontic appliances.**

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Key words: orthodontic appliances, saliva, anthranilic acid.

Introduction

One of the most interesting compounds, which is supplied with food to organism, is tryptophan (TRP). This essential amino acid is making use of protein biosynthesis in human body. 94% of tryptophan is metabolized via kynurenine pathway [1]. Initially tryptophan is transformed into N-formylkynurenine, which immediately is converted into stable kynurenine (KYN) [2]. Kynurenine is precursor of three metabolites such as anthranilic acid (AA), kynurenic acid (KYNA) and 3-hydroxykynurenie (3-HKYN) [3]. Physiological and pathophysological properties of KYNA and 3-HKYN are known very well [4,5]. However, the knowledge about biological role of anthranilic acid in organism is seems to be insufficient.

Recently we have demonstrated the presence of anthranilic acid in saliva of diabetic patients with hypertension [6]. In the present literature is not any information about the role of anthranilic acid in oral cavity pathological state.

Saliva, the secretion of small and big salivary glands, presents fluid environment ecosystem contains: 99.5% of water, 0.3% of organic components and 0.2% of inorganic components. The development of analytic methods has allowed to make discoveries about numerous compounds, which are responsible for physiochemical and biological properties of saliva. The saliva composition is changing in different local and systemic diseases and reflects many pathophysiological states [6]. Moreover, the presence of many of these substances may be a marker of pathological changes in oral cavity and also in general disorders. Since the saliva is easy to get and therefore it can be used as a non-invasive diagnostic tool.

The aim of our study was to estimate the concentration of anthranilic acid in orthodontic appliances. We also assessed the correlation between saliva anthranilic acid concentrations and length of orthodontic treatment.

Material and methods

Nine patients (5 female + 4 male), aged 12-30, with orthodontic apparatus were included in the study. All patients were clinically stable and free of any illnesses. All patients were treated in Department of Orthodontic Medical University of Białystok.

Thirteen (4 female + 9 male) healthy volunteers, aged 18-25, without orthodontic apparatus served as the control group.

Saliva sampling and anthranilic acid determination

Samples of non-stimulated, mixed saliva were taken from studied subjects each morning between 7-8 am, 10 min after mouth washing MilliQ water. The saliva samples were immediately treated 2 M $HClO_4$. After 15 min of incubation with acid at 4°C, samples were centrifuged 30 min 12000 g and the supernatant was collected in -80°C for measurement of anthranilic acid concentration using HPLC method [7].

Statistical analysis

The values are expressed as the mean ± SEM; n – represents the number of results. Statistical analysis was done using Student's t-test. P value less than 0.05 was considered statistically significant. Correlations were analyzed using Pearson test.

Ethics

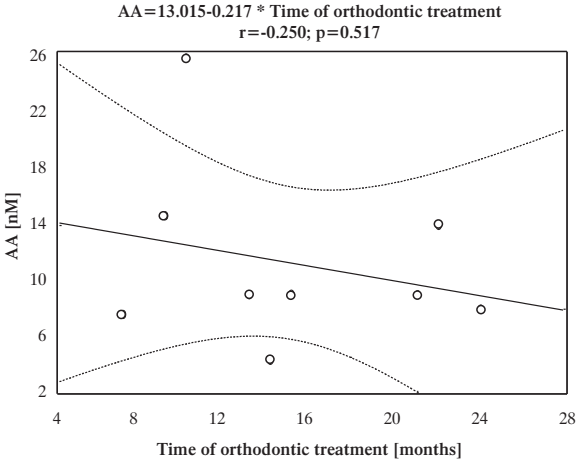
The Ethics Committee of the Medical University of Białystok accepted the study.

Table 1. **Baseline characteristic of orthodontic patients in comparison with control group**

	Control group	Orthodontic patients
Age (yrs)	19.85±0.65	20.0±2.17
Male/female	9M/4F	4M/5F
Time of treatment [month]	-	15.0±2.03
AA concentration [nM]	3.90±1.89	9.75±1.77*

* $p<0.05$ in comparison to control group

Figure 1. **The correlation between anthranilic acid concentration and time of orthodontic treatment**



Results and discussion

The study parameters are summarized in *Tab. 1*. In the control group saliva concentration of anthranilic acid was 3.90±1.89 nM, and increased significantly to a value 9.75±1.77 nM ($p=0.043$) in the group of patients with orthodontic apparatus. The mean time of orthodontic treatment was 15.0±2.03 months. We did not observe existence of correlation between anthranilic acid concentration in saliva and time of orthodontic treatment (AA=13.015-0.217 x time of orthodontic treatment; r=-0.250; p=0.517) (*Fig. 1*).

To our knowledge, this is the first report, which concerns an effect of orthodontic apparatus on anthranilic acid concentration in saliva of young people. In the current study we have observed an increase of anthranilic acid level in saliva during of orthodontic therapy. However, we did not note existence of correlation between anthranilic acid concentration and time of orthodontic therapy. We have believed that this result depends on small group of subjects taken into consideration.

Anthranilic acid is an important, aromatic intermediate in the degradation of tryptophan in kynurenine pathway. For many years it has been know that microorganisms are able to use it as the sole source of carbon and energy aerobically or anaerobically [8]. Now we have known that anthranilic acid plays a key role in the regulation of immunological processes [9,10] and shows antibacterial activity [11].

Our previous study has shown that there is a strong association between anthranilic acid concentration and anaemia in patients with chronic renal diseases [12]. We have proved that this compound can penetrate cell's membrane. Furthermore, we have noticed the existence of negative correlation between anthranilic acid concentration and number of red cells, haematiocrit, and haemoglobin concentration, as well positively relationship between anthanilic acid concentration and osmotic resistance of erythrocytes. In vitro study plays on incubation of healthy subject's erythrocytes with growing concentration of anthranilic acid showed the decrease in resistance of red cells [12]. Its presence in saliva does not indicate the origin of it. On the one hand anthranilic acid can be synthesized by local gingival cells, on the other hand its can penetrate from the blood. Regardless of anthranilic acid origin its presence in saliva is proved in our study.

In conclusion, for the first time we have demonstrated the enhanced concentration of anthranilic acid in saliva of young orthodontic appliances. These results might indicate that anthranilic acid can be one of many factors initiating of periodontal disease in these patients. Thus, further studies are needed to assess whether this increase of anthranilic acid concentration plays an important role in periodontal diseases. Additionally, this study has shown that saliva can be used as a non-invasive diagnostic tool for many pathological state of oral cavity.

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12. Tankiewicz A, Pawlak D, Pawlak K, Szewc D, Mysliwiec M, Buczko W. Anthranilic acid – uraemic toxin damaged red cell's membrane. Int Urol Nephrol, 2005; 37: 621-7. Table. 1. Baseline characteristics of orthodontic patients in comparison with control group.

Periodontitis as a risk factor of coronary heart diseases?

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Abstract

Background: Unstable atherosclerotic plaque is a dangerous clinical state, possibly leading to acute coronary deficiency resulting in cardiac infarction. Inflammatory factor's role in creating pathological lesions in the endothelium of coronary vessels is frequently raised. This state may be caused by bacteria able to initiate clot formation in blood vessel and destabilizing atherosclerotic plaque already present. Source of these pathogens are chronic inflammatory processes occurring in organism, among them periodontal disease as one of more frequent. Aim of the work was to evaluate incidence of selected anaerobic bacteria in subgingival plaque and in atherosclerotic plaque in patients treated surgically because of coronary vessels' obliteration.

Methods: Study was performed on 20 individuals with chronic periodontitis. Subgingival plaque was collected from periodontal pockets deeper than 5 mm DNA test was used for marking eight pathogens responsible for periodontal tissues destruction. In the same patients, as well as in 10 edentulous individuals material from atherosclerotic plaque was collected during by-pass implantation procedure, and identical DNA testing occurred.

Results: In 13 of 20 patients pathogens most frequent in severe chronic periodontitis were found in coronary vessels. In 10 cases those bacteria were also present in atherosclerotic plaque. Pathogens linked with periodontal disease were also found in 7 of 10 edentulous individuals. Most frequently marked bacteria were: *Porphyromonas gingivalis* and *Treponema denticola*.

Conclusions: It seems that advancement of periodontal disease does not have influence on bacteria permeability to coronary vessels. Important is the presence of active inflammatory process expressed by significantly higher bleeding index in patients with marked bacteria in atherosclerotic plaque.

Key words: dental plaque, atherosclerosis.

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The incidence of acute cardiac syndromes (ACS) is closely associated with atherosclerotic plaque destabilization as the result of local inflammatory processes occurring its interior. Inflammation in patients at risk for ACS is manifested as elevated serum concentrations of inflammatory mediators such as C-reactive protein, fibrinogen, serum amyloid, or interleukin 6 (IL-6) [1]. For many years, attempts have been made to identify the microorganisms responsible for the progression and maintenance of this inflammatory reaction. In the 1990's several microorganisms were isolated from atherosclerotic plaque structures and identified: *Herpes simplex virus*, *Cytomegalovirus*, *Mycoplasma pneumoniae*, *Helicobacter pylori* and *Chlamydia pneumoniae* [2-5]. The last pathogen triggers macrophage activation resulting in increased secretion of proinflammatory cytokines, such as interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6). It also increases thrombogenic activity through activation of the thrombogenic system and disruption of fibrinolysis. All of these factors may be the cause of atherosclerotic plaque destabilization that may result in ACS [6,7].

The role of inflammatory factors in the etiopathogenesis of destructive changes in periodontal structures is unquestionable and has been studied for many years [8]. It is known that Gram-negative bacteria in developing dental plaque tend to overpopulate saprophytic Gram-positive microflora. They are a source of enzymes, toxins and many other metabolites directly or indirectly causing connective tissue and alveolar bone destruction.

Lipopolysaccharides (LPS), endotoxins released as the result of bacterial cell membrane fragmentation, are factors triggering the cascade of processes leading to further destruction. LPS cause macrophage activation, which results in the release of IL-1β, TNF-α, prostaglandin E_2 (PGE_2) and proteolytic enzymes – metalloproteinases (MMP's). Released cytokines acting on connective tissue cells (fibroblasts, neutrophils) stimulate them for further production of MMP's. The increase of the PGE_2 concentration activates osteoclasts, leading to destruction of the alveolar bone, while MMP's directly cause the destruction of extracellular connective tissue [9-12].

It seems reasonable to pose the question: is this immuno-inflammatory activity of bacteria limited to the periodontal environment, or can it influence, directly or indirectly, distant structures of the body? Recent evidence suggests that chronic infectious diseases increase atherogenesis and the risk of acute cardiac syndromes, and periodontitis is a persistent bacterial infection causing chronic inflammation in periodontal tissues.

Aim

The aim of the study was to evaluate the incidence of selected anaerobic bacteria in subgingival and atherosclerotic plaques of patients treated surgically because of coronary vessel obliteration.

Material and methods

The study group consisted of 30 individuals with a mean age of 57 years, hospitalized for coronary vessel obliteration at the Clinical Department of Cardiac Surgery, Medical University of Warsaw. These patients were qualified and prepared for by-pass procedures. In the study group, 10 individuals were edentulous, in 20 individuals severe generalized chronic periodontitis was diagnosed (more than 30% of dental pockets affected with clinical attachment loss – CAL), at least two pockets with a depth exceeding 5 mm).

Anamnesis and clinical examination was performed in all subjects, including detailed periodontal examination in patients with preserved teeth. The number of teeth, simplified plaque and bleeding indices, pocket depth (PD), clinical attachment loss (CAL), were recorded.

Bacteriological examination was performed with the use of the DMDx® DNA Test (MicroDenteX). This test is based on DNA hybridization on a nitrocellulose membrane ("slot blot" procedure) and analyzes the incidence of eight selected pathogens in the collected sample: *A.a. – Actinobacillus actinomycetemcomitans*, *P.i. – Prevotella intermedia*, *P.g. – Porphyromonas gingivalis*, *E.c. – Eikenella corrodens*, *C.r. – Campylobacter rectus*, *T.f. – Tanerella forsythensis*, *T.d. – Treponema denticola*, *F.n. – Fusobacterium nucleatum*. In the laboratory, the samples were exposed to a factor causing lysis of bacterial cells, resulting in the release and hybridization of DNA. Material so prepared was placed on a nitrocellulose membrane having the ability to bind denatured DNA. Following this the membrane was exposed to radiologically labeled DNA samples specific for each of the pathogens. If a given pathogen was present in the studied sample, hybridization with radioactive DNA occurred. Autoradiography was then performed with irradiation being proportional to the number of pathogens. An autoradiography film was placed on the membrane and left in the dark for several hours. The developed film was then scanned by video densitometry. The paraquantitative results of the test are presented as follows: negative result (below 10^3 pathogens), low level of bacteria (10^3-10^4 of pathogens in the sample), moderate level (10^4-10^5), and high level of bacteria (above 10^5).

To ensure reliability, the manufacturer suggests verification of patient selection. According to those instructions material was collected from pockets deeper than 5 millimeters, with no suppuration. None of the patients had undergone scaling or taken antibiotics within 6 months prior to the study. No antiseptic mouthwash was used by the patients for 12 hours before sampling.

Selected teeth were cleaned from bacteria and into two pockets deeper than 5 mm sterile paper points were inserted and left for 10 seconds. Points with collected material were inserted into a test-tube, labeled with a bar code exactly the same as the code on the patient's card. Material prepared in this manner was sent to the manufacturer.

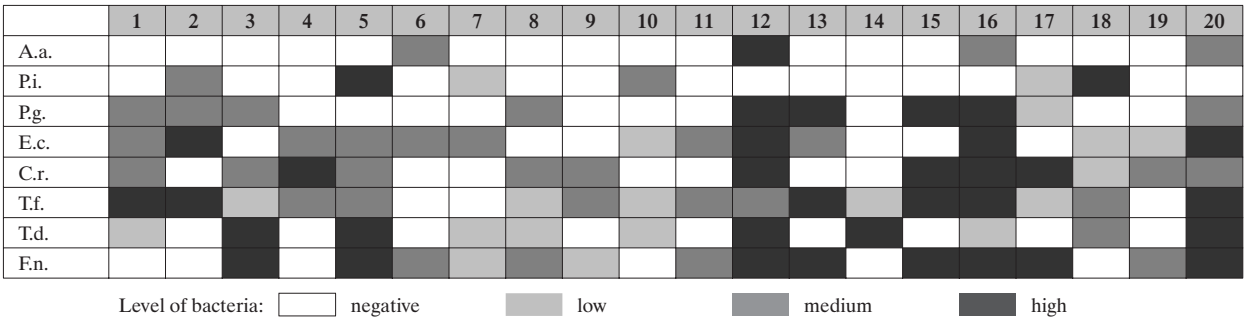
Subgingival plaque was examined in 20 patients with preserved teeth. Atherosclerotic plaque was sampled in all 30 patients during by-pass implantation surgical procedure. Sterile paper points were inserted into atherosclerotic plaque of open coronary vessels and after 10 seconds packed and sent to the laboratory.

To evaluate the degree of advancement of periodontal disease, point classification of periodontal disease it was scored according to Czerniuk; the results are, presented in *Tab. 1* was used according to Czerniuk [13]. Each of 4 examined parameters was evaluated on a numerical scale ranging as the number in the range from 1 to 4, where 1 means low, and 4 – a high value of the measured parameter (a patient with a total of 4 points

Tabele 1. **Numerical scale of advancement of periodontal disease**

Measured parameter	Value of selected parameter	Aquired points
Plaque index	0-25	1
	26-50	2
	51-75	3
	76-100	4
Bleeding index	0-25	1
	26-50	2
	51-75	3
	76-100	4
Pocket depth	0-1	1
	2-3	2
	4-5	3
	6 and more	4
Clinical attachment loss	0-2	1
	3-4	2
	5-6	3
	7 and more	4

Figure 1. **Incidence of bacteria in periodontal pockets**



In columns – patients; in verses – *A.a. – Actinobacillus actinomycetemcomitans*, *P.i. – Prevotella intermedia*, *P.g. – Porphyromonas gingivalis*, *E.c. – Eikenella corrodens*, *C.r. – Campylobacter rectus*, *T.f. – Tanerella forsythensis*, *T.d. – Treponema denticola*, *F.n. – Fusobacterium nucleatum*

Figure 2. **Incidence of bacteria in atherosclerotic plaque**

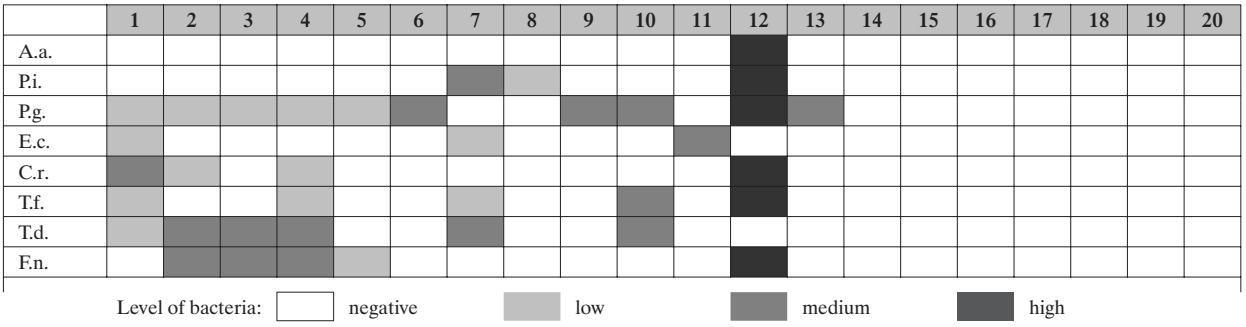


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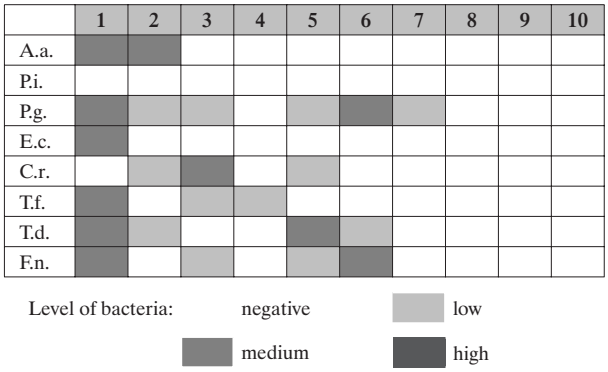
Figure 3. **Incidence of bacteria in atherosclerotic plaque (edentulous patients)**



In columns – patients; in verses – *A.a. – Actinobacillus actinomycetemcomitans*, *P.i. – Prevotella intermedia*, *P.g. – Porphyromonas gingivalis*, *E.c. – Eikenella corrodens*, *C.r. – Campylobacter rectus*, *T.f. – Tanerella forsythensis*, *T.d. – Treponema denticola*, *F.n. – Fusobacterium nucleatum*

generally has a healthy periodontium, an individual with very advanced periodontal disease has got 16 points).

Results

The frequency of selected bacterial pathogens characteristic of severe periodontitis (in 40 pockets of 20 individuals) is presented in *Fig. 1*. In *Fig. 2* the results of bacteriological analysis of material collected from coronary vessels is shown (from the same 20 individuals). In each of 20 patients bacteria regarded as pathogenic for chronic periodontitis were isolated. In 13 of those persons such bacteria were present in coronary vessels; in 10 the same pathogens were present both in periodontium and in coronary vessels.

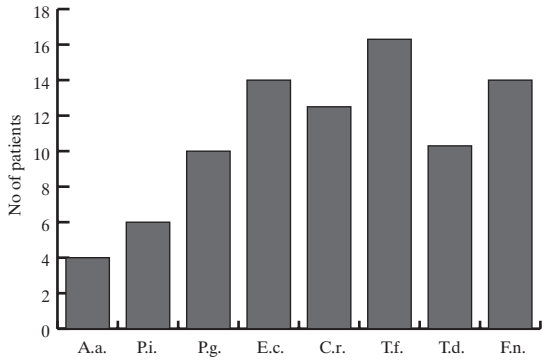
Also in 7 of 10 edentulous patients bacteria responsible for periodontitis were present in atherosclerotic plaque (*Fig. 3*).

To better show the presence of selected bacteria both in the periodontium and coronary vessels, their frequency is presented on *Fig. 4* and *5*. As *Fig. 4* shows, in pockets with a depth exceeding 5 mm, *T. forsythensis* was the most frequent (in 17 individuals), followed by *E. corrodens* and *F. nucleatum* (in 14 individuals), *C. rectus*, *T. denticola* and *P. gingivalis* (in 13, 11 and 10 individuals, respectively).

Fig. 5 presents the frequency of selected bacteria in atherosclerotic plaque of individuals with periodontitis. The most frequent were *P. gingivalis* (in 10 individuals) and *T. forsythensis* (in 6 individuals).

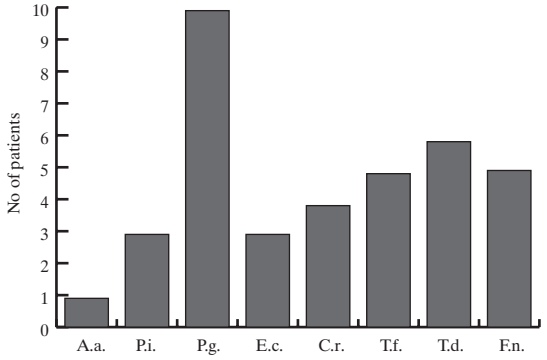
Fig. 6 displays the scores of periodontitis advancement in two subgroups of patients. 13 patients had bacterial pathogens in atherosclerotic plaque, 7 had bacteria present only in the periodontium. Comparison of these two subgroups shows a similar degree of periodontitis advancement (the group with bacteria in the atherosclerotic plaque – 11.77; the group without the those bacteria – 11.57), the slightly difference between the two groups was not statistically significant.

***Figure 4*. Frequency of bacteria in pockets of patients with periodontitis**



A.a. – Actinobacillus actinomycetemcomitans, *P.i. – Prevotella intermedia*, *P.g. – Porphyromonas gingivalis*, *E.c. – Eikenella corrodens*, *C.r. – Campylobacter rectus*, *T.f. – Tanerella forsythensis*, *T.d. – Treponema denticola*, *F.n. – Fusobacterium nucleatum*

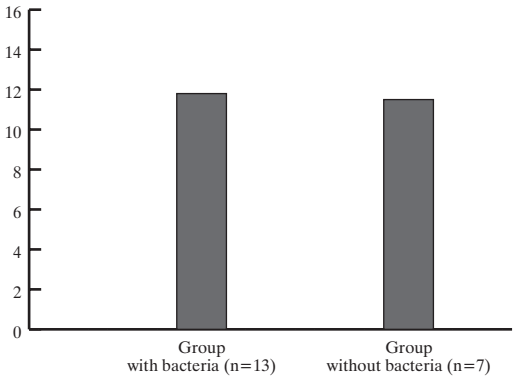
***Figure 5*. Frequency of bacteria in arterial plaque of patients with coronary disease**



A.a. – Actinobacillus actinomycetemcomitans, *P.i. – Prevotella intermedia*, *P.g. – Porphyromonas gingivalis*, *E.c. – Eikenella corrodens*, *C.r. – Campylobacter rectus*, *T.f. – Tanerella forsythensis*, *T.d. – Treponema denticola*, *F.n. – Fusobacterium nucleatum*

Periodontitis advancement expressed as the mean of each clinical parameter measured in both subgroups is displayed in *Tab. 2*. No difference was found in the number of preserved teeth between both subgroups, i.e. between – groups with and without bacteria atherosclerotic plaque (10.69 and 12.71, respectively). The mean percentage plaque index was high in both subgroups, higher in the subgroup without bacteria in atherosclerotic plaque (77.92% vs 93.00% in the other subgroup). There was no statistically significant difference in pocket depth and or clinical attachment loss between subgroups with and without bacteria in atherosclerotic plaque (3.19 mm vs 3.79 mm and 5.53 mm vs 5.69 mm, respectively). The only statistically significant difference was found in the mean percentage bleeding index ($p<0.005$), where a higher mean value was in patients with bacteria in atherosclerotic plaque (55.54%) compared with patients without bacteria in coronary vessels (31.00%).

***Figure 6*. Advancement of periodontal disease in correspondence to bacteria incidence in atherosclerotic plaque**



Discussion

Recent years have brought much research related to the potential connection between periodontitis and coronary disease. Many authors have demonstrated such a relationship; among others in the USA, De Stefano et al., basing on 14 years of research on 9 760 individuals aged 25-74 years, reported an increased risk of coronary disease in the group with periodontitis (25% higher) [14]. Also Beck et al. showed a possible connection between periodontitis and coronary disease. There may also be an association with increased mortality. The authors recorded the periodontal status in 1 147 individuals subjected to 20-year-long observation. In the group with advanced periodontal disease there was an increased risk of severe cardiac episodes and cerebral stroke (1.9 and 2.8 times, respectively) compared with the group without periodontitis [15]. Finnish authors have attempted to evaluate the relationship between chronic tooth-related infections, expressed as the number of lost teeth, and coronary disease. They showed that the incidence of coronary disease in individuals who lost less than a half of their teeth is 10% higher, rising to 2-fold higher in the group that lost more than a half of their dentition. The number of teeth lost proved to be a significant risk factor of coronary disease incidence, but only in smokers [16].

It has been hypothesized that Gram-negative bacteria can migrate from pathogenic dental plaque to the bloodstream, passing through the inflammatory, damaged epithelial attachment. The epithelial lining of the periodontal pocket, which frequently becomes thin and ulcerated in the course of periodontitis, may then provide an entry point for bacteria of subgingival plaque (regardless of their importance to periodontal disease) to gain an access to the underlying tissues and – eventually – to the vasculature. Spreading with the blood, they can affect inflammatory cells present in atherosclerotic plaque and stimulate a cascade of processes leading to atherosclerotic plaque instability [17-19].

The presented study does not unequivocally prove this theory, because bacteria were less frequent in atherosclerotic than in dental plaque, and there was no regular correlation between amount of bacteria in the subgingival pocket and in coronary vessel.

Tabele 2. **Mean values of parameters in relation to bacterial incidence in atherosclerotic plaque**

	Group with bacteria n=13			Group without bacteria n=7			p-level Mann--Whitney
	Mean	Median	SD	Mean	Median	SD	
Age	59.3077	57.0000	9.22302	55.0000	55.0000	6.87992	0.392879
No of teeth	10.6923	9.0000	7.33013	12.7143	12.0000	5.18698	0.274923
Plaque index	77.9231	79.0000	17.19720	89.1429	93.0000	6.81734	0.114628
Bleeding index	**55.5385**	**56.0000**	**21.60099**	**31.0000**	**34.0000**	**12.08305**	**0.006347**
Pocket depth (PD)	3.1923	3.1000	0.68369	3.7857	3.4000	1.09458	0.274923
Clinical attachment loss (CAL)	5.5308	5.4000	1.99348	5.6857	5.2000	1.53886	1.000000

On the other hand, our data does not exclude the bacterial theory, since *P. gingivalis*, *T. forsythensis* and *T. denticola* were found in the material collected from cardiac vessels; these bacteria are intimately related to chronic periodontitis. It is possible that a major role is played by *P. gingivalis*. Madianos showed its presence and ability to reproduce in the epithelium of the periodontal pocket [20], whereas Ishihara showed an increased level of *P. gingivalis* in atherosclerotic plaque, which was correlated to its presence in subgingival pockets [21]. Our own research corroborates this data, as *P. gingivalis* in material from atherosclerotic plaque was the most frequently isolated microorganism in our study. The role of this pathogen in the formation of atherosclerotic plaque is confirmed by research conducted on animals. It proved that periodontitis experimentally generated by *P. ginigvalis* causes greater accumulation of fat in the aorta compared with the control group [22].

Pussinen's research confirms the relationship between periodontitis and cardiac diseases. He showed a higher incidence of cardiac disease in seropositive *P. gingivalis* patients than in seronegative ones (14.0% vs 9.7%). He also reported a higher frequency of coronary disease in individuals with a higher level of antibodies (17.4% vs 11.1%). When discussing the results of linear regression models showing an association of combined antibody response (both for *A. actinomycetemcomitans* and *P. gingivalis*) with the incidence of coronary heart disease (direct) and serum HDL concentration (inverse), he suggests that periodontitis may impair reverse cholesterol transport [23].

Given the ability of *P. gingivalis* to multiply in the epithelium, its ability to penetrate into the bloodstream, the incidence of *P. gingivalis*-stimulated antibodies and role of this pathogen in thrombocyte aggregation through the presence of platelet aggregation-associated protein (PAAP) on its surface, it seems that *P. gingivalis* may play a significant role in the initiation of pathological processes in coronary vessels [24]. The lack of a correlation between the presence of *P. gingivalis* in subgingival and atherosclerotic plaque may be the result of immune reactions and elimination of the bacteria, both through the "first line of defense" (granulocyte infiltration) and specific reactions (humoral and cellular responses). It is possible that permeation of bacteria to the heart results from a disordered immunological response.

Our study also showed the presence of bacteria in atherosclerotic plaque regardless of edentulism. This confirms the findings of Pussinen, who reported a higher incidence of cardiac disease in edentulous patients (19.8% vs 12.1%) [23]. The observed results are difficult to interpret. They may indicate that pathogens present in atherosclerotic plaque could have infiltrated coronary vessels while the patients still had teeth (atherosclerotic plaque formation is a long-term process). The role of bacteria present in nasopharyngeal mucosa and removable prostheses can not be excluded, since they also can permeate through damaged epithelial tissue into blood vessels.

We also have found the same bacteria both in subgingival pockets ≥5 mm and in atherosclerotic plaque (10 of 20 individuals). The statistically significant correlation between bleeding index and presence of bacteria in atherosclerotic plaque may point indicate that active periodontal inflammation favors their infiltration into coronary vessels.

Ishihara has shown differences in the presence of bacteria in subgingival and atherosclerotic plaques in patients having 4 or more pockets deeper than 4 mm, compared with individuals having less than 4 such pockets [21]. He found higher levels of *P. gingivalis*, *T. denticola* and *A. actinomycetemcomitans* in the former subgroup. *P. gingivalis* was isolated from atherosclerotic plaques in 11 of 51 patients (in our material, from 10 of 20). In 11 of those patients, this bacteria was also isolated from the subgingival plaque in 10 (in our material, from 5 of 10).

Our results seem to confirm the possibility that bacteria associated with periodontitis can permeate into coronary vessels. It therefore seems important to limit this process. To achieve this goal, systematic dental plaque elimination is crucial, as well as elimination of retention points favoring its accumulation. The reduction of bacteria present in supragingival and subgingival plaque may be important prophylaxis of both of periodontal and coronary disease.

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Evaluation of the product based on Recaldent™ technology in the treatment of dentin hypersensitivity

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Abstract

Purpose: The aim of the study was to evaluate the efficiency of GC Tooth Mousse in the treatment of patients with dentin hypersensitivity caused by various factors.

Material and methods: The evaluation was carried out on 101 teeth with dentin hypersensitivity in 13 patients. Patients with gingival recession and exposed dental necks and those with non-carious lesions at the initial stage were selected. The initial examination was to evaluate the intensity of pain inducted by a stream of the air syringe and by probing the tooth surface. It was repeated directly after the preparation application, after 15 minutes, after 1 and 4 weeks.

Results: After the medicine application, the number of teeth reacting with strong or extremely strong pain decreased (from almost 80% to 37.62%). The percentage of teeth reacting with mild pain increased by 15% and the number of teeth which did not react to the cold air stream also increased by 27.72%. The values after 15 minutes were similar. A week later, the percentage of teeth with very strong pain was elevated and so was the percentage of medium pain. On the other hand, the number of teeth without pain and with mild pain decreased twice. After one month the percentage distribution was close to the results obtained after 7 days.

Conclusions:

1. GC Tooth Mousse preparation, based on Recaldent™ technology reveals insufficient effectiveness and short-term therapeutic effect in treating hypersensitivity of dentine.
2. It seems that soothing the pain by GC Tooth Mousse should be regarded rather as an additional remineralizing effect of the medicine.

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Key words: dentin hypersensitivity, Recaldent™ technology, clinical evaluation of the treatment, GC Tooth Mousse.

Introduction

Dentin hypersensitivity is a common problem observed in clinical practice. It is defined as algesic overreaction, which cannot be explained otherwise, to harmless sensory stimuli on exposed dentine [1]. Pain hinders everyday activities, such as teeth brushing, consumption food and drinks of various temperatures, speaking, and even breathing. Dentin hypersensitivity is stated in patients with gingival recession as well as cervical and root exposure due to, most frequently, periodontal diseases, after periodontal and surgical treatment, and in teeth with non-carious lesions. Tooth defects and malocclusion, parafunctions as well as improper tooth brushing are predisposing factors as far as cervical exposure and pain are concerned. Tooth hypersensitivity is frequently complained of by patients after whitening and those with removable prosthesis and appliances, at the place of claps adhesion [2-5].

A hydrodynamic theory is assumed to be the most probable theory concerning dentin hypersensitivity occurrence [6]. Liquid movement in dentinal tubules, influenced by a stimulus, causes sensory nerve irritation in the subodontoblastic plexus. Pain intensity, i.e. the degree of hypersensitivity depends on the condition of dentinal tubule openings. Open tubules release algesic reactions while their blockage abolishes the pain. Thus, the aim of the therapy of dentin hypersensitivity is closing dentinal tubules by e.g. crystal precipitation in tubule lumen or hydroxyapatite melting. Laser therapy, preparations with fluorine, hydroxyapatite, strontium and zinc chlorides, potassium oxalate as well as dental adhesives and glass ionomer cement are used for the treatment [4,7-10].

A material GC Tooth Mousse based on the Recaldent™ technology has been recently introduced to the market [11]. Recaldent™ is a unique complex containing amorphous calcium

Table 1. **Evaluation of GC Tooth Mousse effectiveness – the number and percentage of teeth reacting to cold air and mechanical stimuli**

Examination	Cold Air				Probe	
	0	1-3	4-6	7-10	0	+
Before application	–	21 (20.79%)	43 (42.57%)	37 (36.63%)	89 (88.11%)	12 (11.89%)
After the application	28 (27.72%)	35 (34.65%)	29 (28.71%)	9 (8.91%)	94 (93.06%)	7 (6.94%)
After 15 minutes	23 (22.77%)	41 (40.59%)	31 (30.69%)	6 (5.94%)	96 (95.04%)	5 (4.96%)
After 7 days	12 (11.88%)	28 (27.72%)	49 (48.51%)	12 (11.88%)	94 (93.06%)	7 (6.94%)
After 30 days	7 (16.66%)	8 (19.04%)	23 (54.76%)	4 (9.54%)	40 (95.23%)	2 (4.77%)

Legend: 0 – no pain; 1-3 , 4-6 ,7-10 –VAS scale; + – positive pain reaction

phosphate (ACP) and casein phosphopeptide (CPP), obtained from milk casein. The preparation is recommended in hard tissue remineralization. The manufacturer compares the material to "liquid enamel". CPP-ACP complexes make a strong binding with a biofilm on teeth and form a calcium and phosphate reservoir. They are then incorporated into the surface of enamel and dentine [11,12]. Thus, the medicine restores the mineral balance by strengthening hard tissues, reveals an anti-carious potential, and acts synergistically with fluorine. It is used after tooth whitening, professional tooth cleaning, root planing, and curettage. It is also recommended in dentin hypersensitivity reduction due to its ability to block opened dentinal tubules [11]. Therefore, the aim of our study was the assessment of GC Tooth Mousse effectiveness in the treatment of dentin hypersensitivity due to various factors.

Material and methods

The evaluation was carried out on 101 teeth with dentin hypersensitivity in 13 patients, 10 women and 3 men, aged 23-48 years. Patients complaining of pain due to mechanical stimuli (tooth brushing), thermal (warm, cold) or chemical (sweet or sour food) were qualified to the clinical examinations. The exact assessment of patients' dentition was performed and patients with gingival recession and exposed dental necks and those with non-carious lesions at the initial stage (angular lesion, dental erosion and pathological dental abrasion) were selected. Teeth with problems caused by carious lesions, pulpitis, and non-carious lesions qualified for filling (of depth of more than 1 mm) were excluded from the study [5]. We did not take into consideration teeth with cervical fillings and teeth in direct contact with removable prosthesis.

Before GC Tooth Mousse application, patients answered to questions concerning their complaints (intensity, duration, analgesics), oral hygienic procedures, and nutrition habits, which could intensify the symptoms.

The initial examination was to evaluate the intensity of pain induced by a one second stream of the air syringe and mechanically, by moving the probe on the tooth surface. Patients' sensations were classified according to the 10-degree VAS scale (Visual Analogue Scale) [1,13]. The values from 1 to 3 were determined as the mild pain, 4-6 – medium pain, and 7-10 – unbearable pain. The examination was repeated directly after the medicine application, after 15 minutes, after 1 and 4 weeks.

The preparation GC Tooth Mousse is available as a foam and in 5 flavors. Flavor substances stimulate saliva secretion, which intensifies the effectiveness of the medication [11]. The foam application was performed strictly according to the manufacturer indications: the surfaces of examined teeth were carefully cleaned with zinc oxide with water, the operative area was isolated with cotton wool rollers and the thick layer of the preparation was applied on the surfaces, and left for 3 minutes. Then, the patient was instructed to massage the rest of the foam on the teeth with the tongue for 1-2 minutes, without swallowing and spitting out, and to rinse his mouth. He was also forbidden to eat and drink for 30 minutes after GC Tooth Mousse application. In case of persisting pain, the procedure was repeated after 1 week.

Results

The results were presented in *Tab. 1*. During the initial examination of hypersensitive teeth reaction to the stream of cold air, almost 80% of patients described the pain as strong or very strong, hard to resist. After GC Tooth Mousse application, the number of teeth reacting with extremely strong pain decreased and the percentage of teeth reacting with mild pain increased by 15%. The number of teeth which did not react to the cold air stream (scale 0) also increased by 27.72%. The values after 15 minutes were similar. A week after the first application, the percentage of teeth with extreme pain was elevated (from 5.94% to 11.8%) and so was the percentage of medium pain (from 30.69% to 48.51%). On the other hand, the number of teeth without pain and with mild pain decreased twice. On a check-up after 30 days we could only examine 42 teeth as less patients attended the examination. The percentage distribution was close to the results obtained after 7 days. Most teeth did not react with pain to probing during the initial evaluation (88.11%). The percentage increased to above 93% after the application and maintained at the approximate level during the whole period of studies.

Patients histories were presented in *Tab. 2*. It shows that more than half of patients complained of pain due to dentin sensitivity for several years or months. And about 40% of them had tried to treat the disease before. The majority showed the hygienic and nutritional habits that predisposed to pain reaction: inappropriate technique of tooth brushing, with horizontal moves and abrasive toothpastes (for smokers and whitening) and frequent ingestion of products which have a strong acidifying effect on the oral cavity environment.

Table 2. **Data of ailments, oral hygiene and dietary habits (% of patients)**

Hypersensitivity duration	years 61.54%	months 23.08%	weeks 15.38%
Hypersensitivity factors	thermal 55.31%	chemical 27.65%	mechanical 17.02%
Using of any desensitizers before	yes 38.47%	no 61.53%	
Method of toothbrushing	horizontal 36.84%	circular 52.63%	„roll" 10.52%
Hardness of toothbrush	hard 7.14%	medium 78.57%	soft 14.28%
Toothpaste	daily using 45.00%	sensitive 35.00%	abrasive 20.00%
Frequency of fruits and juice consumption	a few times a day 46.15%	once a day 38.46%	a few times a week 15.38%

The patients assessed GC Tooth Mousse and only 23% described it as pleasant while 30% stated that it was unpleasant and irritating.

Discussion

The initial observation of the medicine reveals that its action is short and most effective in the first days of application. Complete elimination of complaint was obtained directly after the application in approximately 28% of cases. The number of teeth with mild pain also increased markedly. However, despite the drug application, 9% of examined teeth still reacted with unbearable pain to the cold air. After a week, the percentage distribution in particular groups changed significantly and unfavorably as the number of teeth with strong and extremely strong pain was elevated. Despite the repeated application of GC Tooth Mousse after a month, the sensitivity of teeth remained at the similar level.

The literature concerning other preparations, like Seal & Protect, Isodan, Green Or, Gluma Desensitizer points to full effectiveness in 60-96% of examined teeth [2-4,7,14]. Moreover, their curative effect persists for more than a month. Those values exceed that obtained in the case of the foam GC Tooth Mousse. Thus, it can be said that this preparation appeared to be less effective, in comparison to others, in treating ailments connected with sensitive dentine. Perhaps, in order to increase its desensitizing effect, the applications should be repeated in intervals shorter than 7 days. Further studies, on a bigger number of teeth and according to such a design, are needed.

It should be stressed that symptomatic treatment would not cure hypersensitivity in case of maintaining bad hygienic, nutritional, and other customs [3,4,7]. Information gathered from patients revealed certain habits that favor the ailment to be persistent or intensified. It should be included into oral hygienic instructions and patients should be persuaded to change their habits.

Conclusions

1. GC Tooth Mousse preparation, based on Recaldent™ technology reveals insufficient effectiveness and short-term therapeutic effect in treating hypersensitivity of dentine.
2. It seems that soothing the pain by GC Tooth Mousse should be regarded rather as an additional remineralizing effect of the medicine.

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Evaluation of bone loss at single-stage and two-stage implant abutments of fixed partial dentures

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Abstract

Introduction: Fixed partial dentures (FPDs) can be supported on implant abutments only or on single-stage and two-stage implants and teeth.

Purpose: The purpose of this study was a comparative analysis of bone loss at the single-stage and two-stage implant abutments of fixed partial dentures used to restore missing teeth classified as Class I or Class II according to the Kennedy classification.

Material and methods: 32 patients were treated by using 49 FPDs supported on implants and teeth worn for 2-6 years. Bone loss at the implant abutments of FPDs was evaluated by one examiner using a special ruler with a measuring scale and images of implants. Measurements were conducted at 26 single-stage implants and 50 two-stage implant abutments based on panoramic radiographs.

Results: Statistical analysis showed that the mean bone loss at implants after 2 years was 0.70 mm± 0.50. The mean bone loss at implants after 6 years was 1.73 mm±0.41.

The bone loss of the alveolar ridge at the single-stage implants was greater than at the two-stage implants but it was not statistically significant.

Conclusion: Prosthetic treatment of missing teeth classified as Class I or II according to the Kennedy classification with FPDs may result in bone loss less than 2 mm after 6 years.

Both single-stage and two-stage intraosseous implants can be suitable for the implant-prosthetic treatment of patients with alar lack of teeth.

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Key words: fixed partial dentures, intraosseous implants, alveolar bone loss.

Introduction

Alar lack of teeth (Class I and II according to the Kennedy classification) can be treated in the maxilla and mandible with removable dentures or with more comfortable fixed partial dentures. This type od dentures can be supported on implants only or on implants and teeth. The problem of connecting teeth with intraosseous implants in patients with partial loss of teeth is often discussed, because intraosseous implants are attached to the bone in a different way from natural teeth. Theoretical analysis, the clinical experience of the authors of publications and experimental studies either recommend or discourage the use of rigid connections of teeth with implants when fixed partial dentures are used [1-11].

The majority of authors present their results in terms of implant failure, implant survival and treatment success. There are fewer reports on bone loss at the single-stage and two-stage implant abutments of fixed partial dentures used to treat the lack of teeth in the posterior areas of the dental arch classified as the Kennedy Class I and Class II [12-15].

The purpose of this study was to evaluate alveolar bone loss at the single-stage and two-stage implants as abutments of fixed partial dentures used to replace missing teeth classified as the Kennedy Class I and Class II.

Materials and methods

The subjects of clinical observations were 32 patients (15 females and 17 males), aged 25-73 (mean age 52), with 49 fixed partial dentures replacing maxillary or mandibular molars or/and premolars. Each FPD was supported by intraosseous implants and teeth (IAFPD – Implant Assisted Fixed Partial Dentures) and the number of tooth abutments and implant abutments var-

Table 1. **Description of patients using fixed partial dentures (FPDs) supported on implants**

Number of patients		Age	Number of patients with posterior lack of teeth		Number of bridges supported on intraosseous implants and teeth		Number of implant abutments	
Female	Male		Unilateral	Bilateral	On single-stage implants	On two-stage implants	Single-stage	Two-stage
15	17	25-73 (av.52)	15	17	20	29	29	47
32					49		76	

Table 2. **Results of bone loss measurements after 2 and 6 years of using fixed partial dentures**

Measurement intervals	Bone loss at both types of implants				Bone loss							
					at single-stage implants				at two-stage implants			
	N	Mean	Median	SD	N	Mean	Median	SD	N	Mean	Median	SD
After 2 years	76	-0.7	-0.5	0.50	29	-0.71	-0.5	0.56	47	-0.69	-0.5	0.44
After 6 years	50	-1.7	-1.5	0.41	27	-1.83	-2	0.40	23	-1.61	-1.5	0.39

ied (*Tab. 1*). Intraosseous implants of the Osteoplant Implantology System (Poland) were applied in the treatment.

All single-stage Osteoplant-Standard implants (26 abutments) and two-stage Osteoplant-Hex implants (50 abutments) were 3.5 or 4.5 mm in diameter and 9-16 mm in length.

Altogether, the FPDs were supported on 76 intraosseous screw implants and 96 teeth. The implants were inserted in the completely cured bone of the alveolar ridge and loaded prosthetically after 3-5 months.

The tooth abutments of FPDs were vital teeth or teeth endodontically treated. All metal-ceramic fixed partial dentures (IPS Classic of Ivoclar – dental ceramics fused to metal) were retained in the oral cavity by using dental cement. The jaws opposing the ones under examination in 28 patients had either natural dentition or fixed partial dentures supported on teeth. Four patients wore removable partial dentures in the opposing jaws.

Panoramic radiographs of all patients were performed before and after implantation, immediately after the placement of the bridges and during the use of the FPDs (2-year intervals). The apparatus applied was a Soredex Cranex Tome, with a magnification of 130 per cent. In this study the objects of analysis were exclusively panoramic radiographs performed after the placement of the dentures in the oral cavity and after 2 and 6 years of use. Because the length of actual implants and the length of their radiographic images were known it was possible to allow for the error of measurement in the analysis of the radiographs.

A special ruler with images of implants (magnified 130%) and a measuring scale on it was used to analyse the radiographs. The loss of alveolar bone at the implants was determined with the help of a viewbox. Measurements were taken and recorded by one examiner after 2 and 6 years of using the fixed partial dentures. The data were analyzed with the Student test ($p<0.05$).

Results

The fixed partial dentures supported distally on the implants and proximally on the teeth of the studied group did not exhibit any clinical or/and mechanical failures. The bone loss of the alveolar ridge varied both at the single-stage and two-stage implants. A summary of measurements of bone loss at the implants is shown in *Tab. 2*.

The mean bone loss at the implants after 2 years of using fixed partial dentures supported on mixed abutments was 0.70 mm ±0.50 (0.71 mm at single-stage implants and 0.69 mm at two-stage implants) and 1.73 mm ±0.41 after 6 years (1.83 mm at single-stage implants and 1.61 mm at two-stage implants). The bone loss of the alveolar ridge at the single-stage implants was greater than at the two-stage implants but the difference was statistically insignificant. It was observed that bone loss at the implants increased during the use of the FPDs and varied in this group of patients. Because only slight atrophy of the bone of alveolar ridge around the fixed partial dentures supported on implants and teeth was observed after 6 years, both single-stage and two-stage implant abutments can be used to treat patients with lack of teeth according to the Kennedy Class I and Class II classification.

Discussion

Many factors are known to affect bone response at implants, such as the type of implants, the kind of material, surface texture, implant location, anatomic area, surgical procedure and prosthetic treatment [3-5,9-12,14-16].

A varied extent of alveolar bone loss at intraosseous implants in the posterior area of the maxillae and mandible has been observed. Based on a nine-year study, Johansson and Ekfeldt reported that the mean marginal bone loss for the implant abutments of fixed partial dentures was 0.4 mm during the first year after prosthesis insertion and less than 0.1 mm per year in the following years [2]. A three-year observation of tooth-implant units supporting fixed partial dentures by Cardaropoli et al. showed that the mean bone loss was 0.5 mm at the implant and was greater than at the tooth. No differences in the bone changes in the proximal area between the implant and the neighbouring tooth were recorded [3].

Differences in bone loss at implants when fixed partial dentures are used to restore missing teeth according to the Kennedy Class I and Class II classification may be caused by a number of factors. One of them appears to be the posterior location of implant abutments (proximity of muscles of mastication), which in combination with the lack of paradontium at implants may result in unpredictable bone loss in this area.

Based on clinical and radiographic evaluation of bone loss at implants placed at molar and premolar sites, Tawil et al. reported that bone loss over the first year was 0.7 mm and 0.81 mm over a three-year period. Their study demonstrated that there was no significant difference between the bone loss around 5 mm diameter fixtures and adjacent 3.75 mm diameter standard fixtures [16]. Taking into account that finding, we did not examine 3.5 mm and 4.5 mm diameter fixtures separately.

Warren et al. observed during a period of 6-36 months following implant placement that crestal bone loss ranged from 0.0 to 2.1 mm in the posterior region of the alveolar arch [17].

A longitudinal radiographic study of fixed partial dentures supported on implant-tooth abutments conducted by Naert et al. showed that the estimated bone loss for the first 6 months was 0.31 mm per year and was greater in the maxillae than in the mandible. Age and gender did not affect the change in the bone level [4].

Hardt et al. found that during a 5-year examination the mean bone loss in the posterior maxillary segment was 1.7 mm for patients without periodontal disease and 2.2 mm for patients with it [12]. Following a 4-5 year study of bridges supported on implant abutments and tooth-implant abutments, Bragger observed favourable clinical conditions for tooth-implant fixed partial dentures, based on an examination of Branemark implants [5].

Naert, Duyck et al. concluded that there is a positive relation between abutment length and marginal bone level. However, their study showed that bone loss never exceeded 2.2 mm even after 15 years [13]. Following their study of Branemark implants as the abutments of FPDs in the mandible, Lindquist et al. reported a mean bone loss of 0.9 mm after 10 years and 1.2 mm after 15 years of using those dentures [15].

The differences in the results of the clinical and radiological studies of various authors appear to emphasise the complex nature of bone loss at implants and its multi-faceted etiology.

Conclusions

– Alveolar bone loss at the single-stage implant abutments of fixed partial dentures is not statistically different from bone loss at two-stage implants.

– Both single-stage and two-stage intraosseous implants can be suitable for the implant-prosthetic treatment of patients with alar lack of teeth.

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The saliva immunology mechanisms and periodontal status in HIV infected subjects

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Abstract

Purpose: The aim of this study was the evaluation of connection between parodontium determined by using GI and PBI indexes and specific immunity status and non-specific in HIV infected group and in control group.

Material and methods: The study was carried out in the group of 37 patients infected with HIV. Mixed non-stimulated saliva was used for the study. Peroxidase activity was determined using the method by Mansson-Rahemtull. Lysozyme and A, G, M antibodies concentrations were determined with the use of radial immunodiffusion method. The concentration of lactoferrin was determined by using ELISA method. The clinical state of parodontium estimated by means of GI and PBI evaluating quality changes in the gum.

Results: Deterioration of the immunological status of subjects was accompanied by the increase of the values of GI and PBI. The strong negative correlation between GI and PBI and the concentration of lactoferrin and positive activity of the peroxidase in the whole examined population was determined. In the infected group the correlation between the status of gingiva expressed by GI and concentration or activity of examined enzymes and immunoglobulins was not ascertained.

Conclusions:

1. HIV infection is connected to worsening of paradontium status expressed by values of GI and PBI indexes.
2. Paradontium status correlated positively with immunological status of HIV positive subjects.
3. In HIV infected group, no connection between number of IgA, IgG, IgM, concentration of lysozyme, lactoferrin, activity of peroxidase and paradontium status was observed.

Key words: HIV infection, GI, PBI, lysozyme, lactoferrin, peroxidase, IgA, IgG, IgM.

Introduction

HIV infection is a very important risk factor of periodontal disease manifestation [1]. It causes the disturbance of the immunological system, which is progressive deterioration of lymphocytes T CD4, leading to AIDS development. The research indicates, that one in ten infected persons is not aware of HIV infection. Changes within the oral cavity are often first symptoms of the HIV infection. The World Health Organization divided disease processes of the oral cavity in HIV infection into three groups [2-4]. The necrotizing ulcerative gingivitis and periodontitis are assigned to changes strongly bounded with HIV.

Material and methods

The study was carried out in the group of 37 patients (11 women and 26 men, aged 19-65, mean age 32 years) infected with HIV, hospitalized in the Teaching Hospital of Infectious Diseases, the Medical University of Białystok. Patients were divided into 3 groups, according to immune disturbances, and a division criterion was laboratory tests concerning the number of CD4 helper lymphocytes T in peripheral blood. The control group comprised of non-infected individuals, counterpart of the examined group. The patients were informed about the aim of the study and they gave their consent. The study was carried out after the approval of the Bioethic Committee of the Medical University of Białystok.

Mixed non-stimulated saliva, collected using expectoration method in the amount of 3-5 ml 2 hours after meal, was used for

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Table 1. **GI and PBI indexes' values in examined groups**

Examined groups	Number of examined subjects	Gingival Index	Standard deviation ±SD	PBI index	Standard deviation ±SD
Infected (1)	37	1.66	0.86	1.01	0.96
Non-infected (0)	37	0.89	0.73	0.68	0.65
Statistical Analysis ($p<0.05$)		0v1		-	

Table 2. **Values of GI and PBI indexes and the time of infected subjects duration**

Examined groups	Number of examined subjects	Gingival Indexs	Standard deviation ±SD	PBI index	Standard deviation ±SD
Infected <4 years (1)	24	1.69	0.78	1.12	1.06
Infected ≥4 years (2)	13	1.61	1.03	0.81	0.73
Total (3)	37	1.66	0.86	1.01	0.96
Control group (0)	37	0.89	0.73	0.68	0.65
Statistical Analysis ($p<0.05$)		0v1 and 0v2		-	

Table 3. **Values of GI and PBI indexes depending on immunological status of examined subjects**

Examined groups	Number of examined subjects	Gingival Index	Standard deviation ±SD	PBI index	Standard deviation ±SD
CD4<200/μl (1)	8	2.02	0.77	1.36	0.92
CD4 200-499/μl (2)	19	1.63	0.87	1.2	1.06
CD4>500/μl (3)	10	1.45	0.9	0.39	0.45
Control group (0)	37	0.89	0.73	0.68	0.65
Statistical Analysis ($p<0.05$)		1v0 and 2v0		1v3	

the study. Saliva samples were centrifuged, divided into portions 200 μl each, and stored at -80°C.

Peroxidase activity was determined using the method by Mansson-Rahemtull et al. [5]. Lysozyme and A, G, M antibodies concentrations were determined with the use of radial immunodiffusion method, ready-made kits (Human NL Nanorid plate – The Binding Site Ltd., UK, BINARID – The Binding Site Ltd.). The concentration of lactoferrin was determined by using ELISA method (Bioxytech Lactof EIA – Oxis Health Products, Inc. USA). The clinical state of parodontium estimated by means of Gingival Index (GI) according to Löe and Silness and Papilla Bleeding Index (PBI) evaluating quality changes in the gum.

The statistical analysis concerning the differences of examined parameters was performed using the analysis of variances (for the variables of normal distribution) or Kruskal-Wallis test (for other variables). In case of significant difference between groups, post hoc analyses were conducted to compare all pairs of groups using t Student test with Bonferroni alteration/amendment (for variables of normal distribution) or Dwass-Steele-Critchler-Flieger test (for other variables).

Results

In HIV infected subjects, GI index was higher than in control group and corresponded to moderate gingivitis, with gingival erythema, tumour and haemmorrhage. In non infected group, the index value indicated subinflammation with mild gingival discoloration. The difference of GI values in those groups was statistically significant. PBI index reached higher value in HIV infected persons than in control group (without statistically significant difference) (*Tab. 1*).

The value GI index did not change fundamentally along with the extension of infection duration. The visible mild tendency of PBI index value decrease with a duration of the HIV infection was not statistically significant (*Tab. 2*).

The value of the GI index indicated a tendency of increase along with the worsening of the immunity status of infected subjects. Its value indicated gingival subinflammation in examined subjects with the absolute number of lymphocytes CD4 >500/μl increased to moderate gingival inflammation in the group with the number of lymphocytes below 200/μl. Statistically significant differences appeared between the control group and examined infected subjects with the absolute number of lymphocytes CD4 below 499/μl. Data introduced in the table III indicate increase of PBI index value along with the worsening of the immunological status of examined subjects, however, statistically significant differences were ascertained only between groups with highest and lowest number of lymphocytes T CD4 (*Tab. 3*).

In persons, who were treated with HAART, the non-significantly higher value of the GI index in infected group, which was not treated with this method and significantly higher in control group was observed. The HAART therapy did not statistically influenced the change of the PBI index value. Only a little higher value in the infected subjects who underwent therapy was observed (*Tab. 4*).

GI values correlated statistically significantly with activity of peroxidase and concentration of lactoferrin in whole examined population. A strong negative correlation between GI and lactoferrin concentration and positive correlation with peroxidase

Table 4. **GI and PBI index values and HAART treatment**

Examined groups	Number of examined subjects	Gingival Index	Standard deviation ±SD	PBI index	Standard deviation ±SD
Infected treated subjects (1)	28	1.82	0.75	1.06	0.99
Infected non-treated subjects (2)	9	1.17	1.04	0.86	0.90
Control group (0)	37	0.86	0.73	0.68	0.65
Statistical Analysis ($p<0.05$)			1v0		NS

activity was observed. In the infected group the correlation between the gingival status expressed by Gingival Index and the concentration or activity of evaluated enzymes was not observed. In non-infected persons, the clinical manifestation of the paradontium correlated statistically positively significantly with the activity of the peroxidase and negatively with the level IgM.

Values of PBI index received in entire population correlated negatively statistically significantly only with lactoferrin concentration and positively with peroxidase activity.

Discussion

The dental research essentially influence the early diagnosis of symptoms of the HIV infection or worsening of the immunological status of the infected person [7]. It has significant meaning especially in developing countries where an access to laboratory-research is limited [8]. Clinical symptoms of the immunological status worsening can have the significant prognostic meaning.

The specific immunodeficiency as result of HIV infection conjointly with changes in the non-specific immunity finds reflection in paradontium tissues and manifests itself with the high average value of GI and PBI in HIV infected persons in comparison to non-infected. As the infection's duration time proceeded values of both indexes decreased. These observations are confirmed by Yeung et al. research [9] according to infected subjects and Friedman et al. [10] research conducted within the group of drug addicts. Different results were obtained by Tukutuku et al. [11], because the gingival index in examined HIV infected population carried out average 0.42 and it was four times lower than in our research. Simultaneously, this result was better than obtained from our control group. There is almost no information about the connection between the immunological status of examined subjects and the paradontium status. In the accessible literature, this problem was examined by Barr et al. [12]. Their results prove the low significant influence of the immunity status on progress of changes in the paradontium. Our research confirmed these dependences and showed that together with worsening of the immunological status of examined subjects the values of GI and PBI increased. Nittayananta et al. research [13] indicate, that decrease of the absolute number of CD4 lymphocytes T below 200/μl is a significant risk factor of the pathological changes appearance within the entire oral cavity. In our research, we observed a correlation between the value of GI and PBI indexes and lactoferrin concentration and the peroxidase activity in the entire examined population. In the HIV infected group, the differences were not statistically significant. In non-infected persons, GI correlated statistically significantly positively with the activity of the peroxidase and negatively with the IgM level. This relation was not confirmed in HIV infected subjects. Jentsch et al. [14] evaluated the influence of the lactoferrin concentration and activity of lysozyme and peroxidase on the healing process of the paradontium after the surgical treatment in the examined non-infected group. Their results indicate that only the lactoferrin concentration can be an appropriate marker to monitor the paradontium status, what is also confirmed by Fine et al. results [15].

Conclusions

1. HIV infection is connected to worsening of paradontium status expressed by values of GI and PBI indexes.
2. Paradontium status correlated positively with immunological status of HIV positive subjects.
3. In HIV infected group, no connection between number of IgA, IgG, IgM, concentration of lysozyme, lactoferrin, activity of peroxidase and paradontium status was observed.

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The evaluation of lysozyme concentration and peroxidase activity in non-stimulated saliva of patients infected with HIV

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Abstract

Purpose: The aim of the study was the comparison of lysozyme concentration and peroxidase activity in mixed, non-stimulated saliva of HIV-positive patients and healthy subjects.

Material and methods: The study was carried out in the group of 37 patients infected with HIV. The control group comprised of non-infected individuals, counterpart of the examined group. Mixed non-stimulated saliva, collected using expectoration method in the amount of 3-5 ml 2 hours after meal, was used for the study. Saliva samples were centrifuged, divided into portions 200 μl each, and stored at -80°C. Peroxidase activity was determined using the method by Mansson-Rahemtull et al. [14]. Lysozyme concentrations were determined with the use of radial immunodiffusion method, ready-made kits (Human NL Nanorid plate – The Binding Site Ltd., UK).

Results: Higher concentrations of lysozyme as well as peroxidase activity were observed in the group of patients with HIV as compared to the control group, and they were 35.08 μg/ml, 46.74 IU/l, 21.3 μg/ml, 37.73 IU/l, respectively. The difference was statistically significant only in case of peroxidase activity.

Conclusions:

1. HIV infection triggers immune mechanisms, that are manifested by the increase in salivary enzymes responsible for local non-specific resistance.
2. The immunological resistance decrease, manifested by the drop of the absolute number of CD4 lymphocytes T, is compensated by the increase in lysozyme concentration and peroxidase activity in non-stimulated saliva of HIV-positive patients.

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Key words: saliva, HIV, lysozyme, peroxidase.

Introduction

HIV attacks cells with an appropriate receptor on their cellular membrane surface. A molecule CD4 is the best known HIV-receptor.

The absolute number of CD4 lymphocytes T is an essential laboratory parameter to evaluate both the course of the infection and the efficiency of the treatment [1]. The number of CD4 lymphocytes T reflects the progress of the disease and shows the gradual dysfunction of the immune system in the course of HIV-infection [2,3].

The immune system of the oral cavity is a part of the systemic immune system. The interaction processes between non-specific (lysozyme, lactoferrin, and peroxidase) and specific elements of the immune system (A, G, and M immunoglobulins) that facilitate to create and maintain the homeostasis take part in the oral cavity. The development of opportunistic infections is caused by HIV disturbing the balance of the immune system [4,5]. Unlike specific factors, non-specific ones act without previous exposure to antigens. The saliva contains various specific and non-specific immunological components that are antibacterial, antiviral, and antimycotic/antifungal in their activities. Lysozyme and peroxidase are the most important non-immunological defense factors of the saliva.

Lysozyme is a glycoside hydrolase of bacteriolytic activity. Its source are the basal cells, mainly in salivary/sialaden leading out ducts, gingival crevice/sulcus fluid, and leukocytes. Lysozyme present in the fluid is assumed to be the blood serum filtrate [6]. The saliva from the parotid glands contains slight amounts of lysozyme while the sublingual glands secrete relatively more of it. It is produced mainly by the submandibular and sublingual salivary glands and shows antiviral and antimycotic/antifungal properties [7,8]. Lysozyme destroys bacterial cellular membranes by means of hydrolysis of 1,4-β glycoside between N-acetylmuramine acid and N-acetyl-D-glucosamine in pepti-

Table 1. **Lysozyme concentrations in mixed non-stimulated saliva depending on CD4 lymphocyte T number**

CD4/μl lymphocyte number	Number of examined subjects	Lysozyme concentration (μg/ml)	Standard Deviation ±SD
CD4<200/μl (1)	8	49.27	43.34
CD4 200-499/μl (2)	19	27.85	23.3
CD4>500/μl (3)	10	37.48	35.38
Total(4)	37	35.08	31.99
Control group (0)	37	21.3	21.88
Statistical analysis (p<0.05)		NS	

Table 2. **Peroxidase activity in mixed non-stimulated saliva depending on CD4 lymphocyte T number**

CD4/μl lymphocyte number	Number of examined subjects	Peroxidase activity (IU/l)	Standard Deviation ±SD
CD4<200/μl (1)	8	54.37	2.71
CD4 200-499/μl (2)	19	46.84	12.07
CD4>500/μl (3)	10	40.45	10.13
Total (4)	37	46.74	11.13
Control group (0)	37	37.73	11.5
Statistical analysis (p<0.05)		4v0, p<0.001	

dyloglycans [9]. It co-operates with other antibacterial systems (e.g. IgA) and causes bacteria aggregation [10]. The activity of lysozyme is intensified by proteases present in the saliva and the acid environment of the oral cavity modulated by monovalent anions, such as: bicarbonates, fluorides, chlorides, and thiocyanates. Lysozyme limits glucose assimilation by bacterial cells, which leads to lowered metabolism, adhesion, and aggregation [11]. Another property of lisozyme is the ability to bind with microorganism nucleic acids, which is essential as far as the homeostasis of the oral cavity is concerned [12].

Peroxidase is secreted by acinous cells of the salivary glands and it is thought to have the most important role in the oral cavity healthy condition. However, myeloperoxidase, produced by leukocytes, has a greater impact in inflammatory conditions with the presence of dental plaque. Peroxidase, together with naturally occurring thiocyanates (SCN-) and hydrogen peroxide (H_2O_2) in the saliva, is a so-called system of salivary peroxidase [13]. It catalyzes the oxidation of salivary thiocyanates by hydrogen peroxide, whose source are oral microorganisms as well as leukocytes and salivary glands. Hypothiocyanic acid (HOSCN) at the low pH and hypothiocyanic anion (OSCN-) at the neutral reaction are the end products of the reaction. It also oxidizes bromides and iodides to hypobromides and hypoiodides [10]. Additionally, myeloperoxidase catalyzes Cl-oxidation. Both enzymes catalyze oxidation of organic compounds, especially phenols. The reaction products react to a wide spectrum of bacteria, viruses, and fungi that occur in the oral cavity. They inhibit the growth, glucose uptake and the production of acids by *Streptococcus* mutans. Peroxidase prevents H_2O_2 accumulation and potential cytotoxic level using the compound produced in oxidation in the oral cavity by numerous streptoccocal strains and in host cells. On the other hand, organic compound oxidation prevents their mutagenic action.

Material and methods

The study was carried out in the group of 37 patients (11 women and 26 men, aged 19-65, mean age 32 years) infected with HIV, hospitalized in the Teaching Hospital of Infectious Diseases, the Medical University of Białystok. Patients were divided into 3 groups, according to immune disturbances, and a division criterion was laboratory tests concerning the number of CD4 helper lymphocytes in peripheral blood. The control group comprised of non-infected individuals, counterpart of the examined group. The patients were informed about the aim of the study and they gave their consent. The study was carried out after the approval of the Bioethic Committee of the Medical University of Białystok.

Mixed non-stimulated saliva, collected using expectoration method in the amount of 3-5 ml 2 hours after meal, was used for the study. Saliva samples were centrifuged, divided into portions 200 μl each, and stored at -80°C.

Peroxidase activity was determined using the method by Mansson-Rahemtull et al. [14]. Lysozyme concentrations were determined with the use of radial immunodiffusion method, ready-made kits (Human NL Nanorid plate – The Binding Site Ltd., UK).

The statistical analysis concerning the differences of examined parameters was performed using the analysis of variances (for the variables of normal distribution) or Kruskal-Wallis test (for other variables). In case of significant difference between groups, post hoc analyses were conducted to compare all pairs of groups using t Student test with Bonferroni alteration/amendment (for variables of normal distribution) or Dwass-Steele-Critchler-Flieger test (for other variables).

Results

Mean lysozyme concentrations in the saliva of patients with HIV were higher than in controls, however, they did not reach the level of statistical significance. All groups showed a wide distribution of results, which is confirmed by standard deviation of mean lysozyme concentrations (*Tab. 1*). The lowest mean lysozyme concentration was determined in the group of patients with the absolute number of CD4 lymphocytes T at the level of 200-499/μl. The deterioration of immunological condition of the examined group, manifested by CD4 amount<200/μl, was accompanied by the increase in lysozyme concentrations (*Tab. 1*).

Tab. 2 presents the mean value of peroxidase activity in examined groups. HIV+ patients showed markedly higher, confirmed statistically, activity of the examined enzyme as compared to the control group. The immunological condition of particular patients did not affect peroxidase activity in the significant manner but a gradual elevation of its activity was observed together with CD4 lymphocyte T absolute number drop.

Discussion

HIV infection is responsible for the immune system impairment, which leads to the progressive CD4 lymphocyte T damage and, as a result, the development of AIDS. Frequent and persistent opportunistic infections in the oral cavity are its consequence. Both specific and non-specific immune mechanisms participate in the defense against pathogenic influence of microorganisms. The non-specific system of secretory resistance of the saliva has its functions based on proteins, which have antiviral, antibacterial, and antimycotic/antifungal properties (lactoferrin, lysozyme, peroxidase, SLPI, cystatine, histatine, defensine, staterine, proline-rich proteins) [15-17].

Our study revealed that lysozyme concentrations in the saliva of HIV+ patients were non-significantly higher in comparison with the saliva of healthy subjects, which is in accordance with other authors' reports [18,19]. On the other hand, studies by Tsang and Samaranayake showed statistically significant higher concentrations of the enzyme in mixed saliva of infected patients as compared to the controls. Lysozyme concentrations varied depending on the immunological status of examined patients and in patients with HIV (absolute CD4 number below 200/μl) was twice higher than in the controls. According to Müller et al. [18], high levels of lysozyme in the saliva of patients with HIV is a result of increased secretion by parotid gland and not the passive transport of the enzyme from blood. It is confirmed by the increase in lysozyme concentration in a salivary gland, in which stimulated local synthesis is a response to cytokines generated by lymphocytes [20].

Peroxidase is another enzyme responsible for maintaining the balance of oral cavity ecosystem. Our results point to the higher peroxidase activity in non-stimulated saliva of HIV+ patients in comparison with non-infected individuals. In the group of infected patients, a negative correlation between peroxidase activity and the immunological status of the examined group was observed. The results of our study are in accordance with the studies by Vučićević-Boras et al. [20], who showed the significantly elevated levels of peroxidase in patients with AIDS with the comparison to healthy subjects and suggested that is a sign of increased antibacterial saliva activity.

Our study presented higher levels of lysozyme contents and higher peroxidase activity in the saliva of patients with HIV as compared to the healthy group. The parameters were increased together with the lowering of the immunological status, expressed with the absolute number of CD4 lymphocytes. Thus, it confirmed the observations of Laibe et al. [5] that there is a relationship between non-specific and specific immune resistance.

Conclusions

1. HIV infection triggers immune mechanisms, that are manifested by the increase in salivary enzymes responsible for local non-specific resistance.
2. The immunological resistance decrease, manifested by the drop of the absolute number of CD4 lymphocytes T, is compensated by the increase in lysozyme concentration and peroxidase activity in non-stimulated saliva of HIV-positive patients.

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Preliminary evaluation of morphological parameters of the saliva in patients undergoing orthodontic treatment

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Abstract

Purpose: In recent years, many reports have focused on clinical changes in the oral cavity of orthodontic patients, manifested in general inflammation of the mucosa. In order to better understand histopathological alterations in the mouth and the use of easily available diagnostic material, we decided to assess the morphology of salivary cells at different time points of treatment with orthodontic appliances.

Material and methods: The study material included non-stimulated saliva obtained from 21 orthodontic patients and 11 healthy secondary school students (controls). After fixation in 96% ethanol the smears were stained with PAS + hematoxylin or H+E, and using the methods of May-Grünwald-Giemsa and Feulgen.

Results: As revealed by the histopathological examinations of saliva smears, patients treated with intra-oral fixed orthodontic appliances showed morphological changes in oral epithelial cells and in the number of leukocytes as compared to the control group. The changes were most pronounced in the first months of treatment.

Conclusions: The preliminary data indicate that orthodontic patients develop changes in the composition and morphology of salivary cells, the intensity of which depends on the time of exposure to the appliance.

Key words: morphology, salivary cells, orthodontic patients.

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Introduction

Clinical changes in the oral cavity manifested in general gingivitis are frequently observed in orthodontic patients. Changes described in literature usually refer to single cases and are mainly detected by clinical evaluation or sometimes confirmed by basic histopathological investigations of oral tissues [1,2]. The increase noted in the number of patients with masticatory abnormalities treated with fixed orthodontic appliances and their clinical picture seem to justify the necessity for the use of accessory diagnostic methods. The saliva, an easily available material, has not been investigated so far. The composition of the saliva, which constitutes a damp environment in the mouth, depends on a number of factors, including plasma composition. In the saliva, desquamating oral epithelial cells, leukocytes and bacteria are suspended. Besides, it contains various biologically active substances, among which some can act as markers not only of oral but also systemic abnormalities. Taking the above into consideration, as well as to better understand histopathological changes in the mouth, we decided to perform a morphological analysis of cells in the saliva of patients at different time points of exposure to fixed orthodontic appliances.

The aim of the current study was to assess the morphological changes in orthodontic patients and their juxtaposition with different time of exposure to the orthodontic appliance.

Material and methods

Investigations were conducted on the non-stimulated saliva obtained from 32 subjects. The control group consisted of 11 generally healthy and non-orthodontic students of the Catholic Secondary School, aged 15-20 years. The study group included 21 patients with food allergy, aged 15-30 years, treated with intra-oral fixed appliances in the Department of Orthodontics in Białystok. The patients were divided into three groups, according to the orthodontic treatment duration: R1 – up to 10 months

Figure 1. **Mucosal epithelial cells and leukocytes in the saliva of control subjects. Mag. x 200 and x 400**

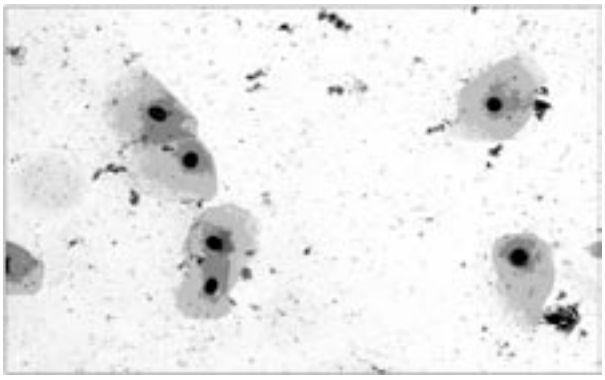


Figure 2. **Mucosal epithelial cells and leukocytes in the saliva of control subjects. Mag. x 200 and x 400**

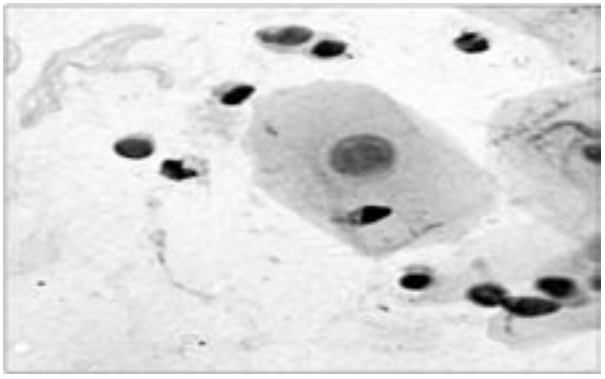


Figure 3. **Varied morphological picture of the epithelial cells and only single leukocytes in the saliva of a patient after a 7-month--exposure to an intra-oral appliance. Mag. x 250**

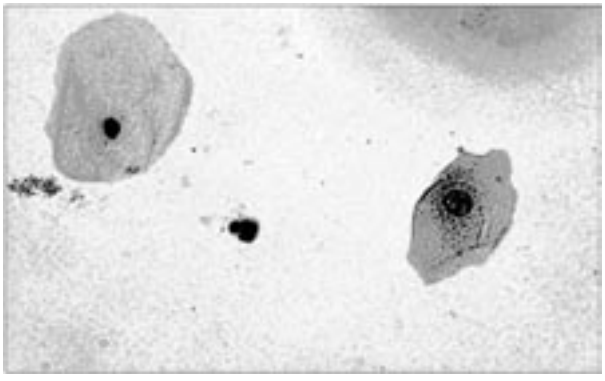
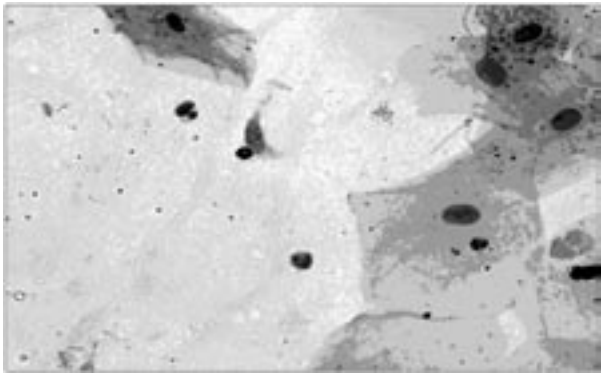


Figure 4. **Morphological picture of the patient's saliva after 24-month-treatment with a fixed orthodontic appliance. Mag. x 250**



(8 patients), R2 – up to 20 months (7 patients), R3 – over 20 months (6 patients).

The study obtained the approval of the Local Bioethics Committee for Research on Humans, Medical University of Białystok.

Each patient gave a detailed history of allergy and other ailments, used medications and accompanying systemic diseases. Then, the status of the oral cavity was assessed by a standard method using artificial light, a mirror and a periodontally calibrated probe.

Saliva smears were done on cleansed and defatted glass slides. After fixation in 96% ethanol the smears were stained with PAS + hematoxylin or H+E, and the methods of May-Grünwald-Giemsa and Feulgen.

Oral epithelial cells and inflow elements underwent microscopic analysis, with regard to shape, size, the nucleus-to-cytoplasm ratio and nuclear chromatin condensation.

Results

In control salivary samples, epithelial cells were numerous, equal in shape and size, and their nucleus-to-cytoplasm ratio was normal and levelled (*Fig. 1*).

Leukocytes, mainly neutrophilic granulocytes and single lymphocytes were observed among epithelial cells (*Fig. 2*).

In orthodontic patients, oral epithelial cells showed morphological alterations. Some of them exhibited changes in the nucleus-to-cytoplasm ratio; others were less regular in shape. Frequently, the cells had a bigger in size, dark pycnotic or vesicular nucleus with loose chromatin. Worth special noting is the presence of characteristic chromatin condensations in the form of rods or serpentine structures throughout the nucleus in some preparations. The morphological changes in epithelial cells were most pronounced in patients in the first (up to 10) months of wearing the appliance (*Fig. 3*). In patients with over 20 months of exposure to the orthodontic appliance, changes in oral epithelial cells were observed only in single preparations (*Fig. 4*).

Smears of the saliva collected after a few months of the exposure to the orthodontic appliance showed a considerably smaller number of inflow cells, as compared to patients after longer treatment duration or control subjects. Only single cells, mainly neutrophilic granulocytes, were sporadically observed (*Fig. 3*).

Discussion

Metals and alloys have a wide range of applications as prosthetic materials for dental tissue reconstruction. The most common metals used in orthodontics are of a composition simi-

lar to 18/8 (18% chromium and 8% nickel) stainless steel and nickel-titanium alloys are widely used in orthodontic treatment [3]. The biocompatibility of dental casting alloys is a critical issue because these alloys are in long-term intimate contact with oral tissues. The release of elements from commonly used dental casting alloys directly into the oral cavity may cause harmful reactions to the host body both locally and systemically [4-6].

The aim of the present study was to investigate the cytotoxicity of materials used in orthodontic appliances by evaluating their effects on changes of cell morphology in the saliva. The results of our study indicate that metal ions released from the alloys can cause adverse effects on cellular metabolism manifested by morphological alterations in salivary cells. In clinical investigations, generalized gingivitis is observed during the first few months of treatment with fixed orthodontic appliances. The mechanisms of these adverse effects have not yet been fully clarified. Some authors have suggested that trace amounts of metal ions can be localized in blood or serum [7], urine [8], and other organs [4,9]. The biological consequences of these released ions on the tissues or cells are still investigated. In order to evaluate the toxicity of the alloys used in orthodontics, an essential approach is to study specific cell populations *in vitro*. Results of the researches have shown that metal ions can reduce cell activity and inhibit specific cellular functions, such as alkaline phosphatase activity [6]. Human gingival fibroblasts exposed to the alloys exhibit alterations in proliferation, glucose-6-phosphate-dehydrogenase activity and in intracellular ATP levels [10-13]. Alterations in cell morphology described in this study can be caused by metal ions binding to endogenous macromolecules, leading to inhibition of various enzymes and having ongoing effects on cell metabolism. In this study, we observed most pronounced alterations in cell morphology in patients who had been undergoing orthodontic treatment for less than 10 months. In this group of patients, we also observed a significantly smaller number of mononuclear cells, perhaps due to cell death. This suggestion is in agreement with those reported by Silvennoinen-Kassinen, who showed that higher concentrations of nickel can induce lymphocyte death *in vitro* [14].

In our study, patients from R2 and R3 groups exhibited slighter metal ions-induced alterations in cell morphology than those with less than 10 months of orthodontic treatment. These data suggest that the longer the treatment continues, the slighter the metal-induced histopathological changes; this in turn suggests that mechanisms of oral tolerance might develop. *In vitro* cytotoxicity tests [1,15] and clinical evidence have been presented to show that small doses of nickel from dental appliances may induce tolerance to this allergen [10,16]. On the other hand, it is likely that metal ions exert a toxic effect only after some time of low-dose exposure. These results indicate that only when the effects of ion release from dental alloys are assessed, extended exposures should be considered.

Further studies are needed to clarify the effects of these metal ions on cellular functions.

Concluding, the current study has demonstrated that release of elements from dental alloys may affect cellular metabolism, which can be manifested by alterations in the number and morphology of salivary cells.

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Preliminary evaluation of saliva composition in allergic patients subjected to orthodontic treatment; morphological examination

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Abstract

Purpose: Intra-oral fixed orthodontic appliances, so frequently used in the treatment of malocclusions, may cause pathomorphological changes in the mouth and can be a potential source of antigen stimulation. Therefore, the aim of the current study was to assess the changes in salivary cells of orthodontically treated allergic patients.

Material and methods: The study material was the non-stimulated saliva samples collected from 28 allergic patients subjected to orthodontic treatment with intra-oral fixed appliances and from 11 healthy secondary school students (controls).

After fixation in 96% ethanol, saliva smears were stained with PAS + hematoxylin or H+E, and using the methods of May-Grünwald-Giemsa and Feulgen.

The microscopic analysis was made of oral epithelial cells and inflow elements, with regard to their shape, size, the nucleus-to-cytoplasm ratio and nuclear chromatin condensation.

Results: The results of preliminary investigations indicate that allergic patients with fixed orthodontic appliances exhibit changes in the morphology and composition of salivary cells as compared to control patients. Differences in the morphological picture were most pronounced in the first months of orthodontic treatment.

Conclusions: It was shown that the number and morphology of salivary cells in allergic patients altered in response to ions released from dental alloys. Thus, saliva can be used as diagnostic material.

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Key words: allergic patients, salivary cells, dental materials.

Introduction

In the last few years, the number of patients subjected to orthodontic treatment has shown a considerable increase and so has the number of scientific reports demonstrating the presence of generalized gingivitis and skin and mucosal reactions leading to tissue inflammation when fixed orthodontic appliances are used [1-6]. Orthodontic appliances are a potential source of ions of various metals released directly to the mouth. The most common metals used in orthodontics are chromium and nickel (stainless steel components) [7]. Because nickel can sensitize certain people and cause severe allergic reactions, the safe use of this alloy is still under study. The knowledge of side-effects, such as irritation, allergies and other toxic effects of long-term contact of the appliance with the adjacent tissues is still incomplete. Most researches investigating toxic effects of orthodontic materials have been conducted *in vitro* on a chosen cell population [7-9]. *In vitro* cytotoxicity tests have been common because they are faster, less expensive, and pose fewer ethical concerns than animal or clinical usage tests. However, considerable controversy remains about the relevance of *in vitro* cytotoxicity tests in the light of studies which have compared them with animal and usage tests [10]. In fact, *in vitro* the cells are free of the general influence of the organism and adapt to the particular conditions of the microenvironment in which they are maintained. Furthermore, in orthodontic therapy, different materials are used and subjected to a damp oral environment, which can modify their properties. The liquid environment of the oral cavity is the saliva, which apart from various biologically active substances contains mucous epithelial cells and numerous blood morphotic elements. Saliva as a diagnostic material is easily available and frequently underestimated. Detection of certain substances in the saliva can be a marker of pathological changes, not only in the mouth but also in the whole body.

Since the mechanisms behind many of the adverse effects have not been fully clarified, the knowledge of the composition of the materials, their irritative and allergenic properties, and other possible toxic effects is essential for their proper and safe use.

Therefore, the purpose of the current study was to assess the changes in salivary cells of allergic patients treated with fixed orthodontic appliances.

Any potential changes in the composition of the morphological activity of salivary cells can confirm the usefulness of saliva as a diagnostic material.

Material and methods

Investigations were conducted on the non-stimulated saliva obtained from 39 subjects. The control group consisted of 11 generally healthy and non-orthodontic students of the Catholic Secondary School, aged 15-20 years. The study group included 28 patients with food allergy, aged 15-30 years treated with intra-oral fixed appliances in the Department of Orthodontics in Białystok. The patients were divided into three groups, according to treatment duration: A1 – up to 5 months (8 patients), A2 – up to 20 months (14 patients), A3 – over 2 years (6 patients).

The study obtained the approval of the Local Bioethics Committee for Research on Humans, Medical University of Białystok.

Each patient gave a detailed history of allergy and other ailments, used medications and accompanying systemic diseases. Then, the status of the oral cavity was assessed by a standard method using artificial light, a mirror and a periodontically calibrated probe. Saliva smears were done on cleansed and defatted glass slides. After fixation in 96% ethanol, the smears were stained with PAS + hematoxylin or H+E, and using the methods of May-Grünwald-Giemsa and Feulgen.

Oral epithelial cells and inflow elements were analysed microscopically, with regard to their shape, size, the nucleus-to-cytoplasm ratio and nuclear chromatin condensation.

Results

The saliva smears of control subjects showed a relatively uniform picture of cell morphology. Epithelial cells were even, usually oval in shape and equal in size. The nucleus-to-cytoplasm ratio in these cells was normal. Among the epithelial cells, leukocytes, mostly neutrophilic granulocytes and single lymphocytes, were present (*Fig. 1*).

In allergic patients treated with fixed orthodontic appliances, the microscopic picture of epithelial cell morphology was less homogenous than in controls. Cells varied in shape, size, in the nucleus-to-cytoplasm ratio and in colour intensity of cell structures. These changes were most pronounced in subgroups A2 and A3. Differences were also observed in the number of mononuclear phagocytes and lymphocytes. Group A1 saliva smears showed numerous neutrophilic granulocytes, monocytes and lymphocytes (*Fig. 2*). The number of inflow cells in the saliva of group A2 patients was markedly smaller as compared to controls (*Fig. 3*). Even fewer inflow cells were found in group A3 – these were mainly "old" neutrophilic granulocytes with nuclei defragmented into numerous lobes, and rare lymphocytes (*Fig. 4*).

Figure 1. **Control salivary smear. The morphological picture of oral mucous epithelial cells is quite uniform. Mag. x 400**

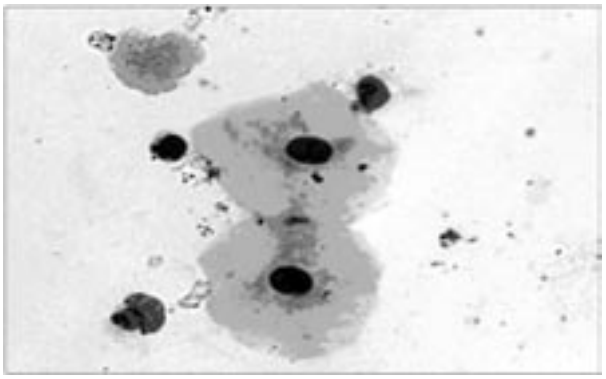


Figure 2. **Numerous neutrophilic granulocytes, monocytes and lymphocytes in the saliva of an allergic patient after 4 months of treatment with a fixed orthodontic appliance. Mag. x 250**

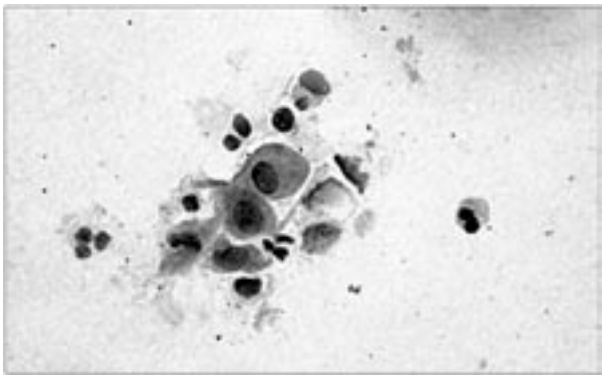
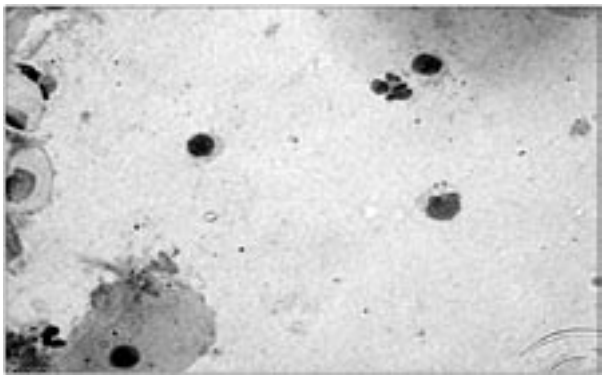


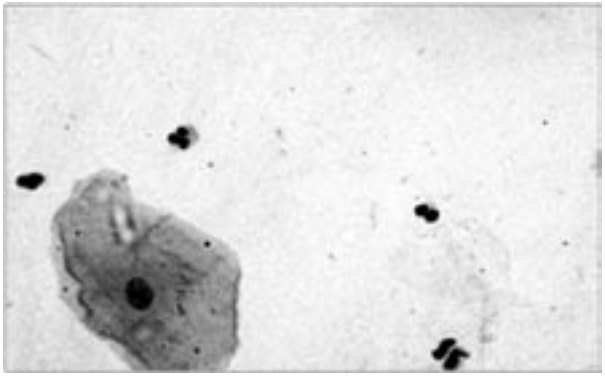
Figure 3. **Saliva cell picture of an allergic patient after 17 months of orthodontic treatment. Mag. x 250**



Discussion

Nickel is a constituent of many dental alloys, which in many people are in long-term intimate contact with oral tissues and

Figure 4. **Saliva smear of an allergic patient after 26 months of treatment with a fixed orthodontic appliance. Mag. x 250**



can induce nickel sensitivity. Clinically, nickel released from these alloys has been shown to cause adverse tissue reactions [11-14]. Reactions of the mucous membranes, such as stomatitis, gum hyperplasias, cheilitis, labial desquamation, and multiform erythemas are frequently noted [15]. Although several studies have reported normal morphology, ultrastructure, viability, and DNA synthesis [16-21], others have demonstrated decreases in DNA, RNA, and protein synthesis, intracellular ATP levels, and inhibition of various enzymes of cultured cells when exposed to nickel-based alloys [8,22,23].

The biologic consequences of released metal ions on tissues or cells have been extensively studied *in vitro*. However, a major problem is associated with relevance of *in vitro* cytotoxicity tests in the light of studies which have compared them with animal and usage tests. The lack of correlation between *in vitro* tests and clinical experience is probably related to many factors [24]. Therefore, in the diagnosis of dental alloy sensitivity it is a great challenge to discover and develop alternative *in vitro* assays to improve and minimize false-positive results.

In our study, patients who had been undergoing orthodontic treatment for less than 5 months showed more evident metal ions-induced alterations in epithelial cell morphology and in the number of mononuclear cells than those with extended exposure. These data indicate that intra-oral exposure to metal ions from orthodontic appliances may have ongoing effects on cellular metabolic functions manifested by morphological alterations. Our data suggest that the longer the treatment continues, the slighter the ions-induced alterations in cells; this in turn suggests that mechanisms of oral tolerance might develop in this context. The mechanisms by which metal ions act in cells are unknown. However, the current results indicate that the concentrations of metal ions which are known to be released from dental materials are potentially capable of altering cell metabolism as well as cellular proliferation [6,15,25,26]. Several investigators have reported that the cytotoxicity of dental alloys may be substantially different after a several months' exposure to a biological medium than after a short time (72-168 h) [6,26]. Studies with nickel-chromium alloys over 35 days [27] showed that the rate of release decreased with time. Clinical evidence has been presented to show that small doses of nickel from dental appliances may induce tolerance to this allergen [28]. Immunologic tolerance to nickel was described by Vreebur, when oral administration of nickel induced partial tolerance in guinea pigs with a splint fixed to their teeth or receiving nickel in their food [29].

In conclusion, it was shown that the number and morphology of salivary cells in allergic patients altered in response to ions released from dental alloys. Thus, saliva can be used as diagnostic material.

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The effect of temperature, acidification, and alkalization changes as well as ethanol on salivary cathepsin D activity

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Abstract

The activity of salivary cathepsin D undergoes inactivation at the temperature of 50-60°C and at pH of 2.0 and pH of 8.0-10.0. The enzyme activity is also decreased by high concentrations of ethanol and high-proof alcoholic beverages. The factors should be taken into consideration in the evaluation of salivary cathepsin D activity.

Key words: human saliva, cathepsin D, temperature, pH, ethanol.

Introduction

Cathepsin D occurs in human saliva [3]. The changes of its activity in the saliva can play an important role in the pathobiochemistry and diagnostics of salivary gland, gingiva, and oral mucosa diseases. External physical and chemical factors can affect the activity of this protease in the saliva.

The aim of the study was to determine the influence of high and low temperature, acidification, and alkalization as well as ethanol and alcoholic beverages on the activity of mixed saliva cathepsin D.

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Material and methods

Ethanol, (POCh Gliwice); hemoglobin (Difco Laboratories, USA); alcoholic beverages: moonshine (home-made), Napoleon cognac (French product), EB beer (Elbląg brewery), Herbowe, Magnat, Porter, Złote beer (Dojlidy brewery – Białystok), Pałacowy brandy (Polmos, Białystok) apple, currant, strawberry wine (home-made), Premium, Zbożowa, Żubrówka, Żytnia vodka (Polmos, Białystok). Alcohol content in alcoholic beverages was determined using an alcoholometer kit [2]; Folina and Ciocalteau reagent (Merck, Germany); copper reagent, prepared according to [1]; other reagents – POCh, Gliwice.

The mixed saliva was collected in fasting state from 12 adults (6 women and 6 men), it was not centrifuged and stored in -75°C. Before the examination, particular saliva samples were mixed with the use of a flow homogenizer. The content of proteins in the saliva was mean 2.8 mg/ml and pH was 6.5.

1. The influence of temperature on salivary cathepsin D activity

The saliva (2 ml) was incubated at 10, 20, 30, 40, 50, 60, 70, and 80°C for 10 min. After incubation, the saliva was cooled with water and ice and pH was brought to 3.5. The amount of 0.1 ml of 6% hemoglobin was added to 0.4 ml of the sample and incubated for 6 hours at 37°C. The reaction was interrupted by adding 0.5 ml of 10% trichloroacetic acid. The samples precipitated at time 0 were considered to be the controls. The content of acid-soluble hemoglobin degradation products was determined in supernatant fluid obtained by centrifugation [4].

2. The influence of pH on salivary cathepsin D activity

The saliva (2 ml) was brought to 2.0-10.0 pH with divisions every 1.0 of pH unit with the use of 0.1 mol/l of HCl or NaOH and incubated at 37°C for 10 min. After incubation, the samples were brought to 3.5 of pH. The procedure was completed as in point 1.

Table 1. **The influence of 10 min incubation of the saliva at various values of temperature on cathepsin D activity**

Temperature, 0°C	Cathepsin D activity	
	Tyr, nmol/l	%
0.0	248.0 ±20.4	100.8
10.0	245.0 ±23.6	99.6
20.0	249.0 ±20.8	101.2
30.0	246.0 ±20.6	100.0
40.0	216.2 ±20.1	87.9
50.0	140.0 ±15.2	56.9
60.0	8.0 ±0.9	3.3
70.0	0.0	0.0
80.0	0.0	0.0

Table 2. **The influence of 10 min acidification and alkalization of salivary cathepsin D**

pH	Cathepsin D activity	
	Tyr, nmol/l	%
2.0	9.8 ±1.2	4.2
3.0	98.0 ±10.4	42.1
4.0	233.0 ±23.1	100.0
5.0	228.9 ±25.4	98.2
6.0	224.6 ±24.2	96.4
7.0	220.0 ±21.1	94.4
8.0	140.4 ±12.0	60.2
9.0	18.6 ±2.0	8.0
10.0	0.0	0.0

Table 3. **The effect of various concentrations of ethanol on salivary cathepsin D activity**

Ethanol % w/v	Cathepsin D activity	
	Tyr, nmol/l	%
0.0	186.4 ±19.8	100.0
0.625	185.2 ±18.6	99.4
1.25	180.4 ±17.4	96.8
2.5	152.6 ±15.0	81.9
5.0	120.2 ±13.1	64.5
10.0	92.6 ±9.8	49.7
20.0	86.0 ±9.1	46.1

Table 4. **The effect of alcoholic beverages on salivary cathepsin D activity**

Beverage	Ethanol concentration, % w/v	Cathepsin D activity	
		Tyr, nmol/ml	%
Control	0.0	186.2 ±19.6	100.0
Moonshine	7.9	96.4 ±10.2	51.8
Napoleon cognac	4.8	132.0 ±11.8	70.9
EB beer	0.9	184.5 ±19.2	99.1
Herbowe beer	1.1	188.0 ±18.4	100.9
Magnat beer	1.2	179.8 ±18.0	96.6
Porter beer	1.7	184.6 ±19.2	99.1
Złote beer	1.1	182.6 ±18.8	98.1
Pałacowy brandy	5.9	148.5 ±15.0	79.7
Apple wine	2.5	158.4 ±15.6	85.1
Currant wine	2.3	158.9 ±15.2	85.3
Stawberry wine	1.8	167.4 ±16.0	89.9
Premium vodka	7.8	96.0 ±10.4	51.6
Zbożowa vodka	6.5	124.2 ±13.8	66.7
Żubrówka vodka	5.2	132.0 ±12.0	70.9
Żytnia vodka	6.7	126.0 ±13.6	67.7

3. The effect of various ethanol concentrations on salivary cathepsin D activity

The amounts of 0.1 ml of ethanol of the concentrations from 3.120 to 100% w/v and 0.1 ml of 6% hemoglobin were added to 0.3 ml of the saliva and incubated at 37°C for 6 hours. All reagents were of 3.5 of pH. The procedure was completed as in point 1.

4. The influence of various alcoholic beverages on salivary cathepsin D activity

The amounts of 0.1 ml of an alcoholic beverage and 0.1 ml of 6% hemoglobin were added to 0.3 ml of the saliva and incubated at 37°C for 6 hours. All reagents were of 3.5 of pH. The procedure was completed as in point 1.

Results and discussion

It was observed that the temperature from 0 to 40°C did not affect the activity of salivary cathepsin D (*Tab. 1*). The temperature of 50-60°C significantly lowered the activity of this enzyme while the temperature from 70°C and higher inactivated it. Salivary cathepsin D activity was markedly decreased at 2.0 of pH (*Tab. 2*). The enzyme was stable at 3.0-7.0 of pH whereas at pH of 8.0 and higher – it underwent inactivation. Ethanol in the concentration of 5.0-20.0% w/v lowered the activity of salivary cathepsin D (*Tab. 3*), as did high-proof alcoholic beverages (*Tab. 4*). However, beer and wine did not have any effect on the activity of this enzyme.

The decrease in the activity of cathepsin D at the temperature above 50°C and pH of 8.0-10.0 was also observed incases of the enzyme derived from other sources [5,9]. The degree of salivary cathepsin D activity decrease by ethanol and alcoholic beverages depended on their concentrations [8]. The inhibition of gastric and pancreatic proteolytic enzymes was also observed [6,7,10].

The influence of physical and chemical factors on cathepsin D activity should be considered while determining the enzyme activity in the saliva for the evaluation of the salivary gland functioning as well as the diagnostics of the oral cavity diseases.

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Local argyrosis of oral mucosa or amalgam tattoo. A problem in diagnosis and treatment

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Abstract

The authors, basing on three cases published by different authors in the years 1995-2003, discuss the problem of diagnosis and treatment of local gingival argyrosis and amalgam tattoo. Treatment methods carried out consisted of the following procedures free gingival graft, subepithelial connective tissue graft in a two-step procedure and subepithelial connective tissue graft without flap coverage. In the authors opinion in some cases a connective tissue graft does not need flap coverage, therefore a dual blood supply is not necessary.

Key words: amalgam tattoo, argyrosis, treatment of pigmentations.

Introduction

Available literature of the past 10 years presents cases of gingival pigmentation, caused by the presence of silver particles. Surgical procedures with the use of free gingival graft or connective tissue graft were most often incorporated in treatment [1-3]. These publications show some vagueness and disagreement particularly concerning diagnosis and methods of treatment. In this article worth looking into information has been gathered and discussed.

Rusch-Behrend and Guttmann [1] in 1995 presented a case of gingival argyrosis in a 26-year-old woman. According to the authors this lesion is also referred to as amalgam pigmentation or amalgam tattoo. The lesion was located on the upper gingiva overlying central incisors. Radiographic examination showed that both central incisors were treated endodontically. The root canals were obturated with a silver cone and an amalgam root-end restoration. The authors suggested that gingival pigmentation was caused by silver particles released from the silver cones and amalgam restorations as a result of material corrosion.

Carried out treatment consisted of removal of the prosthetic crowns, removal of silver cones and amalgam restorations. The root canals were refilled with thermoplasticized gutta-percha. A full-thickness mucoperiosteal tissue flap was reflected and curettage of the osseous tissue along with thinning of the reflected tissue was carried out. Root-ends were resected.

Histological analysis of biopsied tissues revealed chronic inflammatory cell infiltrate predominantly plasmocytic and multiple aggregates of black foreign material, which was recognized as silver containing salts also localized in granulation tissue.

To further reduce tissue argyria four weeks following endodontic surgical intervention periodontal grafting procedures were performed. A full-thickness free gingival graft from the palate was placed at the site of the discoloration. After 8 months healing was completed but pigmentation was still observed localized in the vestibule above the lateral incisors, a whitish scar formed between the pigmented gingiva and facial mucosa.

Kissel and Hanratty presented a different method of treating gingival metallic discolorations [2]. These authors proposed a total surgical removal of pigmentation from the underlying gingival connective tissue and coverage by a subepithelial connective tissue graft from the palate. The graft was placed between the periosteum and the overlying partial thickness gingival flap.

Two and a half months post surgery a thin partial thickness flap was performed along the same incision lines and removed. Clinical examination following further 2½ months after the second surgery showed complete rekeratinization of the subepithelial connective tissue and achievement of ideal gingival color matching the surrounding tissues. The technique incorporated

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Figure 1. **Discoloration of the gingiva to superior teeth**

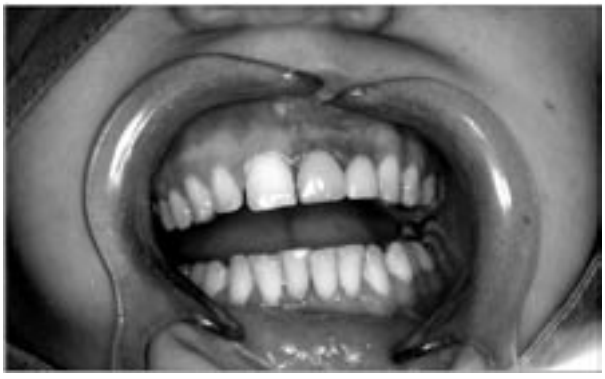


Figure 2. **Directly after removing of discoloration of the gingiva**

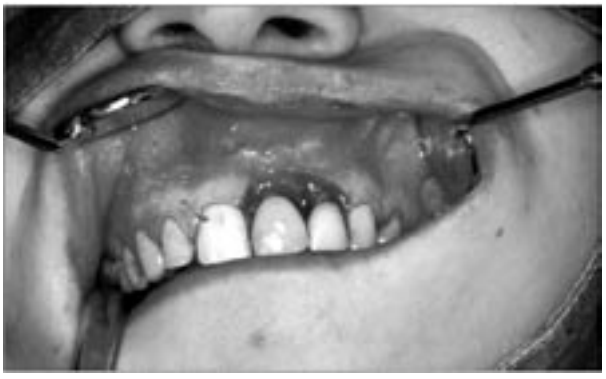


Figure 3. **Palatal discolored gingival was kept intact**

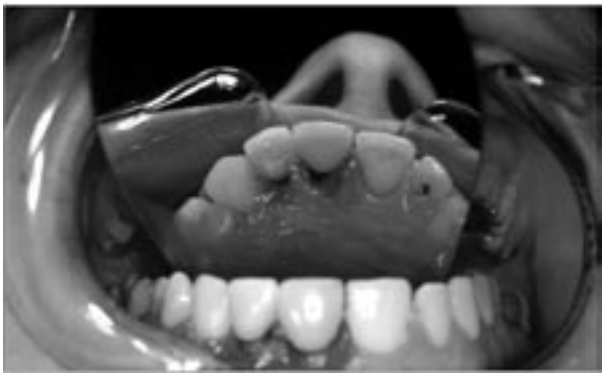


Figure 4. **Subepithelial connective tissue in the region of removed gingiva discoloration**

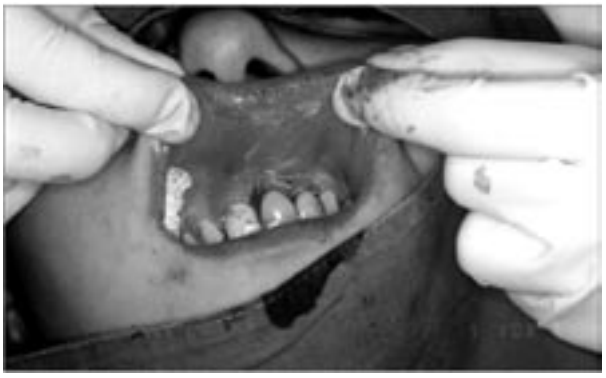
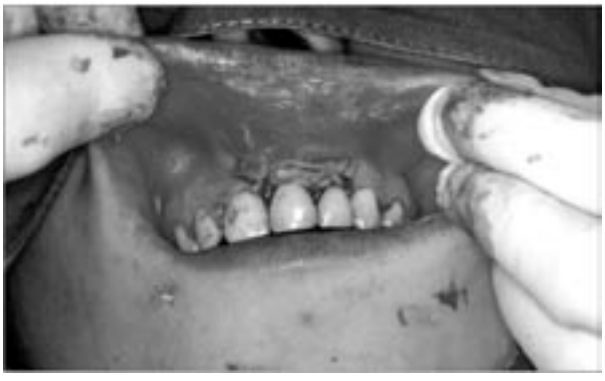


Figure 5. **Subepithelial connective tissue sutured to the regional gingiva**



by Kissel and Hanratty has been described as supraperiosteal ridge replacement technique.

The third case was described by authors of this article [3] and concerned a gray discoloration of the gingiva superior to maxillary left, central and lateral incisors, 21, 22. The discoloration appeared around these teeth 6 years ago after placement of acrylic crowns (*Fig. 1*). The discolored gingiva gave no dental symptoms but on smiling esthetics were disturbed.

The above described discoloration appeared as a result of accidental implantation of silver particles during aggressive preparation of cemented posts and cores probably with the use of a high speed handpiece without irrigation [3]. In January 2003 a surgical procedure was carried out which consisted of excision of the labially discolored gingiva beyond the border of the discoloration and curettage of the osseous and connective tissues covering the roots of both teeth (*Fig. 2*). The gingival papillas between the upper, left, central and lateral incisors and between the central incisors and the left lateral incisor and canine were completely removed. Palatal discolored gingiva was kept intact (*Fig. 3*).

After removal of discolored gingiva root denudation was estimated at approximately 2.5-3.0 mm.

A subepithelial connective tissue graft was harvested from the palate in the region of the right premolars the size of the recipient site (*Fig. 4*). The graft was secured using non-absorbable sutures 7-0 (*Fig. 5*) and care was taken to achieve precise adherence to all margins of the surgical wound. This contact allowed for blood supply to the graft, which was not even partially covered by a gingival flap. The recipient site was covered by surgical cement for a period of 10 days. In the same way the palatal wound was provided.

Healing after 4 months revealed total incorporation of the graft, complete rekeratinization of the subepithelial connective tissue and achievement of ideal color match to the surrounding tissues (*Fig. 6, 7*). The reproduced gingiva healed with the partially left gingival papilla and palatal gingiva. Root denudation was completely covered with produced gingiva,

Figure 6. **4 months after the operation and changing the crowns**

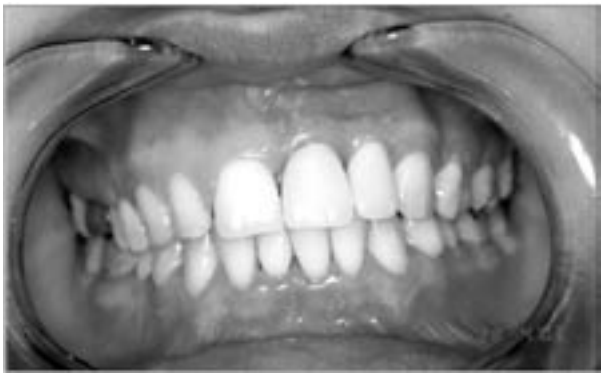
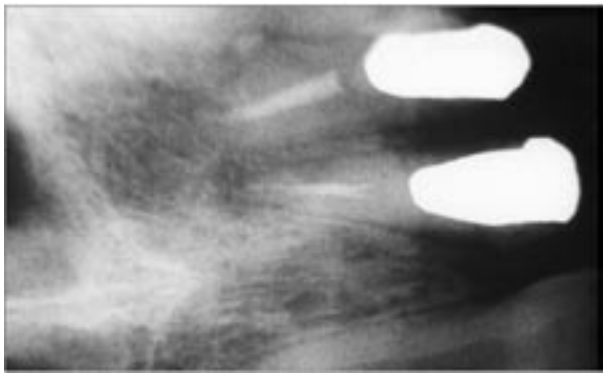


Figure 7. **Silver cones in the root canals of both teeth**



epithelial attachment was probably achieved as probing depth was 1.5 mm. This confirms Harris's reports about regeneration abilities of connective tissue grafts [4].

Discussion

All of the presented cases were caused by different factors and were also treated by different methods.

The first case [1] concerned an amalgam tattoo treated surgically with the use of a free gingival graft placed in the discolored site but results were unsatisfactory.

The second case [2] described a gingival pigmentation above the maxillary lateral incisor it was not certain whether it was gingival argyrosis or an amalgam tattoo. The patient was treated by a two-step surgical method with the use of a subepithelial connective tissue graft. The method was quite complicated but effective.

The third case [3] described gingival argyrosis treated with a subepithelial connective tissue graft without flap coverage. Treatment results were astoundingly effective.

The three presented surgical methods of treating gingival pigmentation show that the least effective seems to be the use of free gingival grafts harvested from the hard palate. These grafting procedures were described by Björn in 1963 [15], although there were earlier reports by Younger in 1902 and Hartland in 1906 [13]. These methods were recommended to manage tissues in case of discolorations [1,2]. However, from the esthetic point of view employment of these grafts in surgical procedures should be rejected, for the reason that color match to surrounding tissues is unsatisfactory [17] (*Fig. 6*).

Rusch-Behrend and Guttmann [1] in their discussion of results of the first presented case mention the disadvantages of free gingival grafts. For the patient treated in their study other periodontal techniques – such as subepithelial connective tissue graft known in the 90s for its esthetic value – were considered inappropriate because of the size of the defect and of the wide dissemination of amalgam particles what could have detracted from the final result.

The use of subepithelial connective tissue grafts, harvested from the hard palate, to widen keratinized gingiva was described in 1971 by Karring and Ellegaard [cit. 16].

In 1980 Langer and Calagna [cit. 2] reported a technique using a subepithelial connective tissue graft to correct ridge deformities caused by extractions. According to these authors this type of graft should be provided with a dual blood supply, from the gingival flap and periosteum. All other authors employing connective tissue grafts are in agreement with this opinion [5,7,13,16-21]. Kissel and Hanratty [2] introduced a special method "supraperiosteal ridge replacement technique" to eliminate amalgam tattoo while maintaining ridge form. They introduced two surgical concepts:

1. replacement of connective tissue containing amalgam particles with subepithelial connective tissue graft eliminating most pigmentation and maintaining ridge form.
2. elimination of remaining epithelial pigmentation by excision of thin partial thickness flap allowing the graft to rekeratinize and achieve an esthetic result.

Results of the third case [3] treated with subepithelial connective tissue graft without flap coverage, are not consistent with other authors opinions concerning the need of dual blood supply to the graft. In this case the graft received blood supply only from gingiva surrounding the recipient site and remnants of periosteum and bone. The graft was placed in the recipient site where blood supply came only from the margins of the incisioned gingiva, the periosteum was removed as biopsy results showed that it contained silver particles. In these conditions for 2-3 days the graft was supplied only by plasmatic circulation [22]. Wilcko at al. [20] in similar cases suggest creating bone penetration to improve blood supply to the connective tissue graft from the bone.

Not all properties of connective tissue grafts have been recognized. Harris [4] emphasizes that these grafts have regenerative properties and can be used to increase the width of keratinized gingival. Zabalagni at al. [21] emphasize that in the case of connective tissue grafts formation of periodontal attachment to root surface occurs but the formation mechanism is not well known. The authors of the third case [3] confirm this. In the carried out study as a consequence of gingival formation the periodontal sulcus depth was 1.0-1.5 mm while root denudation after excision of pigmented gingiva came to 2.5-3.0 mm.

Root coverage could take place primary directly following grafting or secondary with the participation of creeping attachment [21].

The final question needs answering: was there a need for a two-step surgery in the second case published by Kissel and Hanratty [2]? According to the authors this surgical procedure was necessary as coverage of connective tissue graft with a mucosal flap was not possible and only after healing-in of the connective tissue graft it was deprived of epithelial coverage.

In the third case, the epithelium was formed from the connective tissue flap or creeped in from incision margins. According to Harris [4] a connective tissue graft has the ability to form epithelium. Therefore it can be certainly confirmed that in some cases the connective tissue graft can heal-in even with only a unilateral blood supply.

Another problem is the differential diagnosis of gingival discolorations.

Eversole [5] basing on Buchners and Hansens [6] study concerning 268 cases of amalgam tattoo confirms iatrogenic mechanism of forming of these discolorations caused by accidental implantation of amalgam particles in the mucosa during routine dental procedures such as preparation of teeth with amalgam fillings. Terheyden and Kern [7] warn against implantation of metal particles into the gingiva and formation of discolorations during preparation of titanic implants without irrigation.

Other cases presented in literature concern systemic argyrosis resulting from oral application of pharmaceutics containing silver for a long period of time. Such a case was reported by Pardro at al. [8] and concerned a 45-year-old woman who recieved a pharmaceutic containing silver for a period of 25 months. This was followed by argyrosis of skin, nails, hair and mucosa. Another case of systemic argyrosis was described by Marshall and Schneider [9] and occurred as a result of 2½ year local application of silver nitrate in the oral cavity and resulted in discoloration of skin, stomach and duodenum. Haseth at al. [10] reported presence of silver selenide (Ag_2Se) concrements in the skin following several months of treatment of gingival erosions with silver nitrate. After a period of 11 years selen was found in 4 cases of systemic and local argyrosis basing on results of radiographic microanalysis [11].

The problem arising in discussion as a result of the presented cases is differentiation of amalgam tattoo and gingival argyrosis. Differentiation of these changes was studied by Dominiak at al. [12] and resulted in the following conclusions: local argyrosis is only related with silver particles implanted in tissues or released during corrosion of silver while amalgam tattoo contains silver and particles of other metals such as: copper, mercury, tin, iron, selenium, molybdenium and sulphur [13]. Wu at al. (14) in 16 out of 24 examined discolorations found to contain amalgam remnants and only 8 contained other particles: Ag, Hg, Sn and Co. Harrison at al. and Wethers and Fince (cit. 13) basing on examinations in electron microscope and sample microanalysis found that in case of amalgam corrosion silver and tin are absorbed by macrophages and are surrounded by giant cells. Macrophages are migrating cell this could explain the spreading of tattoos. Silver also cumulates in basal membrane of epithelium, smooth muscles of blood vessels, fibroblasts, collagen, elastic tissue and fibers of Schwann's capsule [2]. Unknown is the length of time mercury remains in tissues and what role it can play. Unexplained is the role of electrogalvanic currents and their influence on metal particles migration. According to Kissel and Hanratty [2] particles of silver remaining in tissues attract other silver particles causing enlargement of the tattoo.

Differential diagnosis of amalgam tattoo and gingival argyrosis has not been settled. There are differences in chemical composition of factors causing discoloration and divergent opinions concerning their migration in tissues but it is not resolved whether these findings have influence on treatment efficiency.

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Transient oral cavity and skin complications after mucositis preventing therapy (palifermin) in a patient after allogeneic PBSCT. Case history

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Abstract

Purpose: The aim of this study was to assess the state of oral mucosa in a patient after allo-PBSCT who has received palifermin, a recombinant human keratinocyte growth factor.

Material and methods: A 19-year-old male was treated in the Department of Haematology of the Medical University in Warsaw due to the AML. Conditional chemotherapy was applied, according to the BuCy 4 + ATG regimen and allogeneic haematopoietic cells transplantation from an unrelated donor. He was receiving palifermin intravenously for 3 consecutive days immediately before the initiation of conditioning therapy and after allogeneic PBSCT. On day +3 the oral mucous membrane was pale and swollen, with linea alba visible on cheeks. Superficial glossitis and viral pharyngitis were noted. Beginning with day +5/+6 proliferative gingivitis was observed. On day +9 gingival contour was altered and the gingiva covered nearly completely tooth crowns of all teeth. The gingiva were whitened, as if covered by thick epithelium. Slight gingival hyperplasia was still observed on day +24. Since day +4/+5 skin rash coexisted, spreading over hairy head skin, face, dorsum and chest. Disseminated papulopustular (acne-like) lesions were observed, some of them related to the hair follicles. Skin changes were present till day +15.

Conclusions: Palifermin is an efficient pharmaceutical in mucositis prevention in patients after allogeneic PBSC transplantation. Transient complication of hyperplastic gingivitis with a concomitant skin eczema of a papulopustular nature arose.

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Key words: mucositis, PBSCT, palifermin.

Introduction

Mucositis (the inflammation of oral mucosa) is a frequent adverse events of haematopoietic stem cell transplantation (HSCT). Oral mucous membrane damage is the result of the applied conditioning treatment, i.e. chemotherapy and/or total body irradiation, infections developing as a result of neutropenia and agranulocytosis, as well as teeth injuries [1]. Mucositis is one of the earliest adverse events and arises between 5 and 7 days after the conditional therapy. It is also one of the most devastating. It occurs when cancer treatment destroys the rapidly dividing epithelial cells, particularly in the oral cavity, leaving the mucosal tissue opened to ulceration and infection. Clinically mucositis is characterized by pain, swelling and erythema. Erosions, ulcerations and splits, excessive or decreased saliva secretion and spontaneous bleeding can also occur [1,2]. All these symptoms lead to discomfort, make solid, and often even fluid food ingestion impossible, frequently imposing parenteral nutrition and narcotic analgesics administration. At the same time the damaged oral mucosa with a concurrent neutropenia constitutes a prospective port of entry for life-threatening infections [3,4]. Mucositis affects about 60 to 100 percent of patients after haematopoietic stem cells transplantation [5]. It plays a crucial role in the pathogenesis of post-transplantation complications. It correlates with an increased perioperative mortality rate, longer hospitalization period and the increase of treatment costs.

In 1995 Amgen submitted a Biologics License Application for palifermin, a recombinant human keratinocyte growth factor. Keratinocyte growth factor is a 28-kD, heparin-binding member of the family of fibroblast growth factors that was originally isolated from pulmonary fibroblasts as a protein (FGF-7) with keratinocyte-stimulating activity. Palifermin (recombinant human keratinocyte growth factor) is an N-terminal, truncated version of endogenous keratinocyte growth factor with biologic

Figure 1. **Patient RD on day +9 after PBSCT**

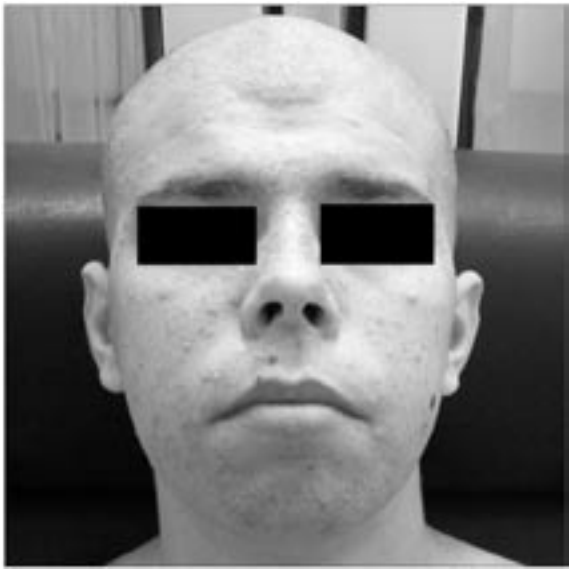


Figure 2. **Patient RD on day +9 after PBSCT**

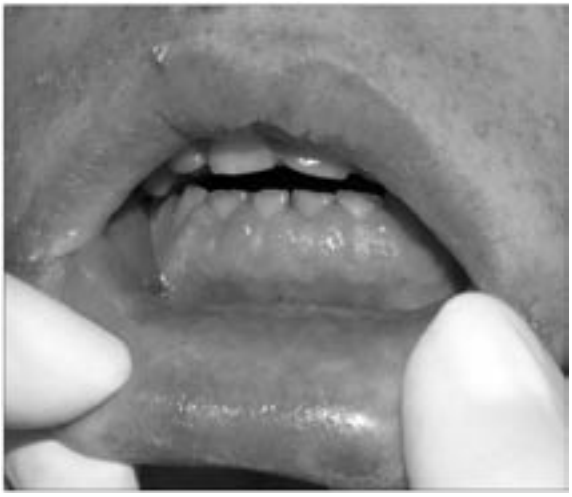
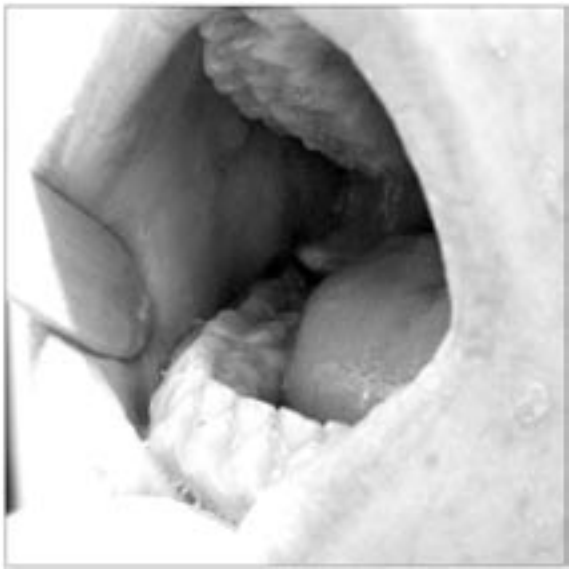


Figure 3. **Patient RD on day +9 after PBSCT**



activity similar to that of the native protein, but with increased stability [6]. Recently, palifermin (Kepivance [Amgen ,US]) was approved as the first cytokine shown to decrease the incidence and duration of severe oral mucositis in patients with haematologic malignancies receiving myeloyoxic theraphy requiring haematopoetic stem cell support [7-9].

Purpose

The aim of this study was to assess the state of oral mucosa in a patient after PBSC allotransplantation who has received palifermin, a recombinant human keratinocyte growth factor.

Material and methods

A 19-year-old male was treated in the Department of Haematology of the Medical University in Warsaw due to the AML with M2 according to FAB. Conditional chemotherapy was applied, according to the BuCy 4 + ATG regimen (busulfan 4 mg/kg/d on days -9 through -6; cyclofosfamide 50 mg/kg/d on days -5 through -2; ATG 10 mg/kg/d on days -3 through -1) and allogeneic haematopoietic cells transplantation from an unrelated donor. He was receiving palifermin (60 microg/kg/d) intravenously for three consecutive days immediately before the initiation of conditioning therapy (on days -13, -12, -11) and after allogeneic PBSCT (on days +3, +4, +5). The clinical assessment of oral mucous membrane state was performed every third day. Pathologic eruptions presenting on the oral mucous membrane were noted. Mucositis was assessed according to the WHO scale. The patients assessed the subjective pain feeling in the 10-point scale.

On day +3 the oral mucous membrane was pale and swollen, with linea alba visible on cheeks. Superficial glossitis and viral pharyngitis were noted. Beginning with day +5/+6 proliferative gingivitis was observed. On day +9 gingival contour was altered and the gingiva covered nearly completely tooth crowns of all teeth. The gingiva were whitened, as if covered by thick epithelium (*Fig. 1-4*). Slight gingival hyperplasia was still observed on day +24 (*Fig. 5,6*). During the forming of gingival hyperplasia the patient had a subjective "membrane growing" sensation with tingling and itching. He reported an oral cavity pain score of 0 in the 10-point pain scale. No mucositis was present (WHO scale = 0). Since day +4/+5 skin rash coexisted, spreading over hairy head skin, face, dorsum and chest. Disseminated papulopustular (acne-like) lesions were observed. Some of them were related to the hair follicles. Skin changes were present till day +15. Neutropenic fever was noted on day +6 (absolute leucocytosis 0.1 G/L). On day +19 after the transplantation the WBC regeneration reaching > 1 000/μl, comprising 0.5/μl neutrophils, was noted. CSA (Neoral, Sandium) in dose of 5 mg/kg body weight on day –1 and 3 mg/kg in the intravenous infusion starting on day 0; Mtx (metotrexat) 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3 and +6 were administered as a prophylaxis

Figure 4. **Patient RD on day +9 after PBSCT**

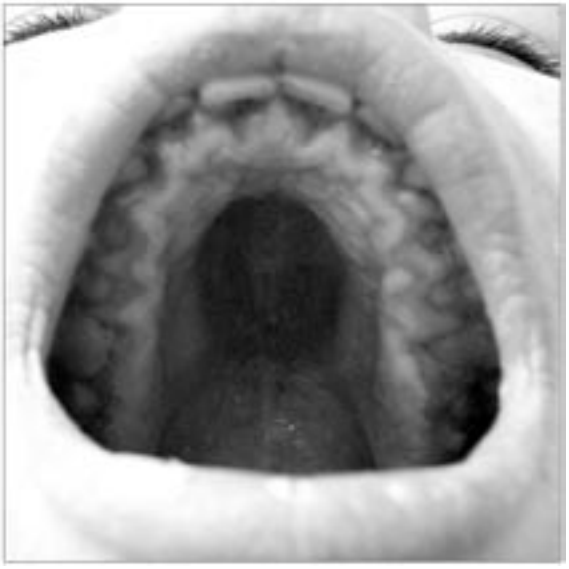


Figure 5. **Patient RD on day +19 after PBSCT**

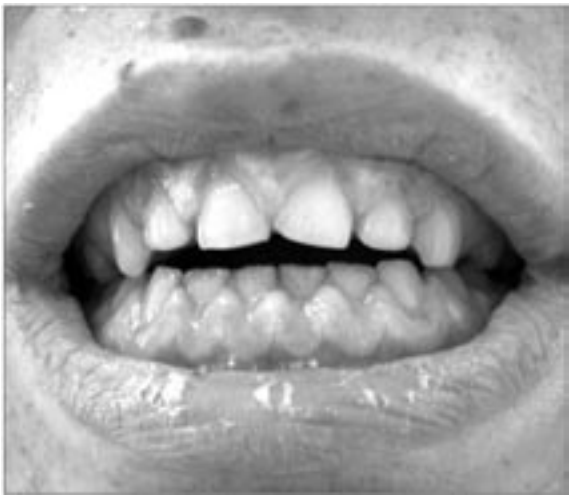
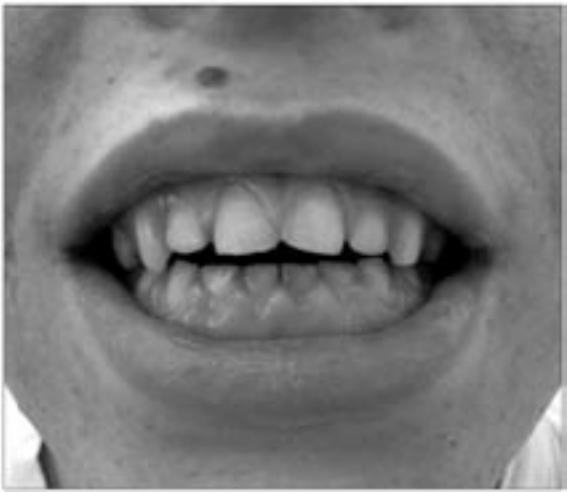


Figure 6. **Patient RD on day +21 after PBSCT**



and treatment of GHVD. On day +15 after transplantation in the postoperative treatment human immunoglobulins (IVIG) were administered in dose of 0.5 g/kg. Concomitant medications were Orungal 2x200 mg p.o., Heviran (aciclovir) 5x400 mg p.o., Tazocin 4x4.5 g i.v. on days +2 and +3, Maxipime 3 x 2.0 g i.v. on days +3 through +7, vancomycin 2 x 1 g on days +5 through +14, metronidazole 3x500 mg i.v. on days +4 through +14, Neoral 2x100 mg i.v. since day +5 and 2x150 mg since day +13, Zyrtec 1 tablet/day since day +6.

Conclusions

Palifermin is an efficient pharmaceutical in mucositis prevention in patients after allogeneic PBSC transplantation. Transient complication of hyperplastic gingivitis with a concomitant skin eczema of a papulopustular nature arose.

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Periodontal condition in patients with cardiovascular diseases

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Abstract

The cardiovascular system diseases constitute a serious problem for modern medicine.

The aim: To investigate the potential risk and the connection of periodontal diseases and cardiovascular disorders.

Material: The examination was performed in the group of 104 patients of both sexes, aged 50-90 years. The patients were divided into two groups: group I – patients with hypertension (47 subjects), group II – patients with fresh myocardial infarction, treated with primary coronary angioplasty (57 subjects).

Methods: The OHI index, according to Greene and Vermillion, was used to assess the oral hygiene and periodontal clinical conditions were evaluated according to Russell's PI index, modified by Davies. CPI index was used to estimate the state of periodontium. Teeth loss was classified according to the Eichner's classification.

Results: The value of OHI index differs in both groups. Highest value was registered at 5 patients in the I group vs 2 in the II group. Lowest value was recorded in 11 patients in the I group and 4 in the II group. The value 0.0-0.2 PI was recorded at 14 persons in the I group and 11 in the II group. The value 1.6-3.8 of PI index was registered at 2 in the I group and 6 in the II group. Healthy periodontium was stated in 10 patients with hypertension and only 2 with myocardial infarction. The CPI=2 was shown in 12 patients with hypertension and 11 with myocardial infarction, CPI=3 was shown in 23 patients with myocardial infarction.

Conclusion: The studies revealed bad condition of the oral cavities of patients with hypertension, and specifically with fresh myocardial infarction.

Key words: periodontal disease, myocardial infarction, hypertension.

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Introduction

The cardiovascular system diseases constitute a serious problem for modern medicine. Despite common beliefs, it is the cardiovascular system pathology that is the most frequent cause of death in the industrial countries and not neoplastic diseases [1,2]. Improper nutritional habits, lack of physical activity, and stress lead to the increase in the risk of the cardiovascular system disorders, i.e. coronary heart disease and hypertension. Death of so-called "myocardial infarction" accompanies people just like loss of teeth. The mortality due to myocardial infarction is very high in Poland and is still increasing.

Cardiovascular diseases are characterized by intravascular and rich in fat deposits, that can induce vascular clots and lead to heart death [3-5].

Age, obesity, lipid disorders, hypertension, and diabetes mellitus are commonly accepted as the risk factors. The studies have been focused on the role of lipids, mainly cholesterol, accumulating on vascular walls and the results revealed inflammatory process activity occurrence, besides cholesterol accumulation. The inflammatory process, taking place in the atheromatous lamina causes its destabilization. The oral cavity infectious focuses can be the source of microorganism dissemination to the bloodstream. Bacterial pathogens from caries focuses of periodontium pass to the bloodstream and can induce changes, not only in the area of periodontium but also in large vessels. Bacteremia frequently leads to the damage of the valves, the layer lining the inside of the heart, and vessels [3,6,7].

Periodontium is a part of the masticatory organ, which is

Figure 1. **Division of patients regarding sex and the type of disorder**

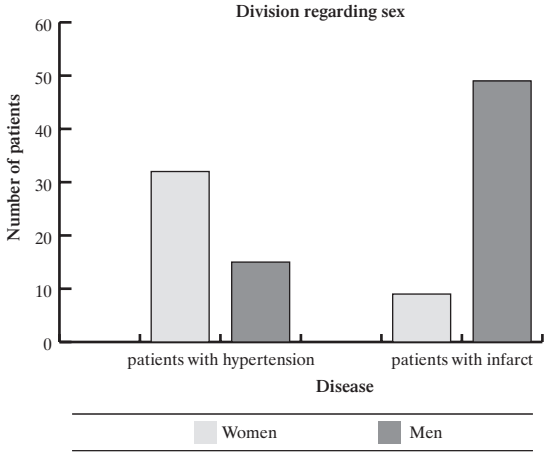
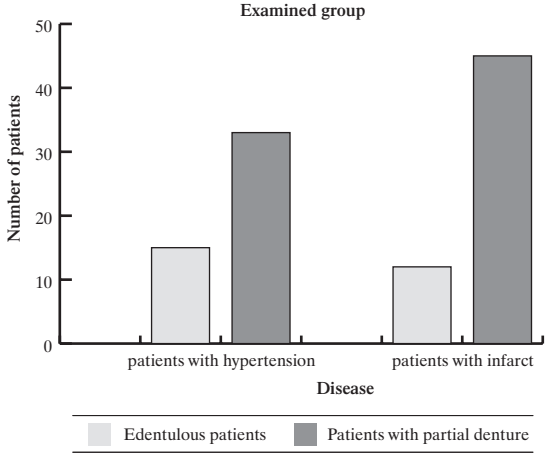


Figure 2. **Tooth loss occurrence in examined group**



composed of tissues that are in contact around the tooth neck, i.e. the gingiva, periodontium, periosteum, root cement, and the bone of the alveolar process. The epithelial attachment is the connection of the tooth and the gingiva at the neck. It plays a crucial role in periodontal physiology and pathology as it constitutes the gingival crevice floor, which normally is from 0.5 to 1.5 mm in depth.

A chronic inflammatory process, taking place in periodontium, causes the epithelial attachment damage. Reddening, oedema, and spontaneous bleeding or bleeding at probing are the symptoms of the inflammatory process in the clinical picture. The factor inducing the occurrence of the inflammatory process is dental plaque, which can cover both the surface of a tooth and the gingiva [8].

Thus, it is important to examine the masticatory organ condition in patients with cardiovascular diseases to determine the potential risk and the connection of periodontal diseases and cardiovascular disorders.

Material and methods

The examination was performed in the group of 104 patients of both sexes, aged 50-90 years. The patients were divided into two groups:

- group I – patients with hypertension (47 subjects)
- group II – patients with fresh myocardial infarction, treated with primary coronary angioplasty (57 subjects).

All patients were hospitalized and gave their written consent to the examination. The study was approved by the Bioethical Committee of the Medical University of Białystok.

A survey was prepared for the study and the information concerned demographic data and the kind of disease. The masticatory organ conditions were assessed in artificial light using a diagnostic set (the mirror and probe) and the periodontological probe.

The OHI index, according to Greene and Vermillion, was used to assess the oral hygiene and periodontal clinical conditions were evaluated according to Russell's PI index, modified by Davies.

CPI index, determining the parodontal condition with code values, was used for the assessment [8]. Teeth loss was classified according to the Eichner's classification.

Results

The examined group consisted of 104 patients, out of which 47 were hospitalized due to hypertension (group I) and 57 subjects – due to myocardial infarction (group II). Group I included 32 women and 15 men while in group II men outnumbered women (49 and 8, respectively) (*Fig. 1*). The majority of both groups showed partial teeth loss whereas the minority revealed total loss of teeth (*Fig. 2*).

The oral hygiene condition in the examined patients was presented in *Fig. 3*.

In 11 patients with hypertension, good oral hygiene was noticed (index=0), 16 patients revealed dental deposit or supergingival calculus covering 1/3 of the tooth surface (index=1), larger amounts of dental deposits (index=2 and 3) were observed in 5 and 2 patients, respectively. Only 4 patients with myocardial infarction showed satisfactory oral hygiene (index=0), 23 patients presented dental deposits or supergingival calculus covering 1/3 of the tooth surface (index=1), bad oral hygiene (index=2 and 3) was noticed in 13 and 5 patients, respectively.

Fig. 4 showed periodontal clinical condition in the patients. Clinically healthy periodontium (values 0.0-0.2) was observed in 14 patients with hypertension and 11 with myocardial infarction. Gingivitis (values 0.3-0.9) was stated in 16 individuals with hypertension and 22 with myocardial infarction. The beginning of periodontitis (values 0.7-1.9) occurred in 2 patients with hypertension and 6 with myocardial infarction whereas advanced periodontitis (values 1.6-3.8) was observed in 2 patients with hypertension and 6 with myocardial infarction.

Periodontal condition according to CPI index in the

Figure 3. **Oral hygiene condition in examined patients**

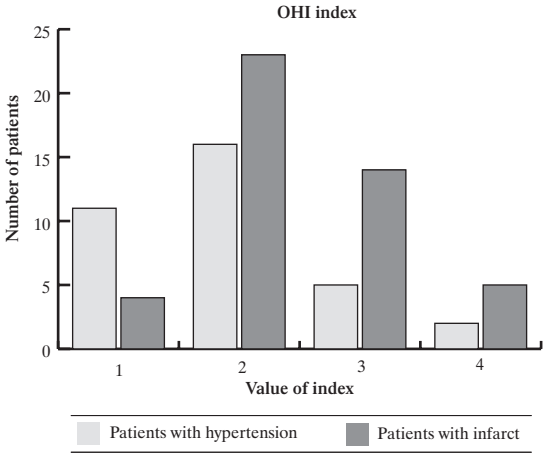


Figure 4. **Periodontal condition according to PI index in examined patients**

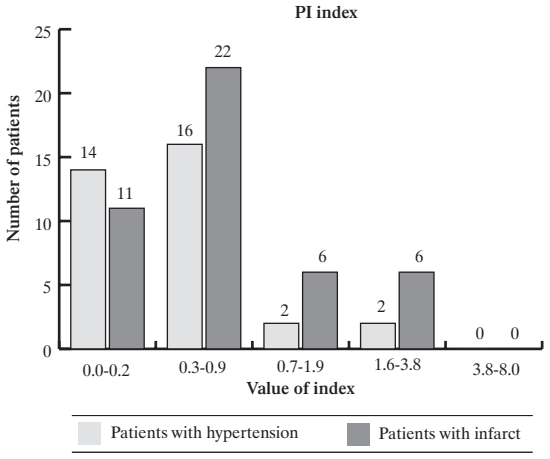
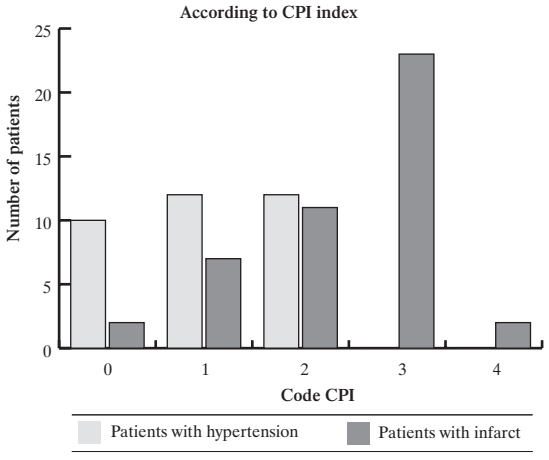


Figure 5. **Periodontal treatment needs in examined group**



examined group of patients was presented in *Fig. 5*. Healthy periodontium that did not require specialist treatment (CPI=0 code) was stated in 10 patients with hypertension and only 2 with myocardial infarction. Gingival bleeding after delicate probing (CPI=1) was noticed in 12 patients with hypertension and 7 with myocardial infarction; super- and subgingival dental calculus, overhanging edges of fillings and crowns (CPI=2) were shown in 12 patients with hypertension and 11 with myocardial infarction. Gingival pockets up to 5.5 mm (CPI=3) were observed in 23 patients with myocardial infarction while deep gingival pockets above 6 mm (CPI=4) were stated in 4 patients with myocardial infarction. Gingival pockets did not occur in patients with hypertension.

Discussion

The studies revealed definitely worse periodontal condition in patients hospitalized due to myocardial infarction than that in patients with hypertension. The majority of patients, specifically with fresh myocardial infarction showed marked periodontological treatment need. We also noticed definitely worse oral hygiene state in patients with fresh myocardial infarction as compared to hypertensive patients.

Many clinical studies point to the significance of the oral hygiene and the consequences of neglected caries and oral inflammatory condition treatment. Each carious defect or improper hygiene of the oral cavity is a potential source of bacteria that can induce infectious endocarditis, myocardial infarction, and cerebral stroke [4,5,7,9].

According to many scientists, long-term exposure to circulating microorganisms due to bacteremia occurring in natural conditions of everyday life, e.g. lack of the oral hygiene, has a crucial role. Therefore, lack of the oral hygiene and neglect of treatment of inflammatory changes are stressed to be more dangerous than the risk of bacteremia during dental procedures as nowadays, antibiotic prophylaxis is recommended as the standard procedure [4,7,9].

Infectious endocarditis is a serious, often fatal disease, with the complications such as the myocardial infarction, cerebral stroke, or circulatory failure. It happens when bacteria are transported to the bloodstream mainly from the oral cavity or other parts of the body [1,4,5].

Olczak et al. [5] stated that periodontal bacterial pathogens could be the cause of infectious endocarditis. A damaged gingival crevice epithelium or pathological pocket epithelium constitute a wide gate for bacteria and bacterial toxins invasion to the bloodstream. Katz et al. [10] in their study concerning patients after the myocardial infarction or diagnosed coronary heart disease observed more frequent, however, statistically insignificant, occurrence of parodontal diseases in over 10.5 thousand of Israeli soldiers. Janket et al. [11] stated, on the basis of epidemiological studies, the increase by 19% in the risk of coronary heart disease and cerebral stroke occurrence in patients with periodontal diseases. According to them, the risk was elevated to above 44% in patients after 65 year of age. Zaremba [7] in his studies revealed severe periodontal in patients with cardiovascular disorders, which occurred twice as frequently as in healthy subjects. Wiśniewska-Spychała et al.

[12] noticed periodontitis in all 107 patients with coronary heart disease qualified for by-pass operations.

Our studies confirmed that periodontal condition in patients with cardiovascular disorders, specifically with fresh myocardial infarction, was unsatisfactory. The marked loss of connective tissue attachment, deep pockets, and numerous dental deposits prove the active periodontal disease, which can have a connection with cardiovascular disorders.

Conclusions

1. The studies revealed bad condition of the oral cavities of patients with hypertension, and specifically with fresh myocardial infarction.

2. There is an urgent need to show the relationship between the oral condition and the cardiovascular system pathology.

3. The co-operation between general practitioners, cardiologists and dentists needs to be intensified.

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Short time effect of elmex® and Listerine® mouthrinses on plaque in 12-year-old children

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Abstract

Purpose: This study was conducted to determine the effect of two mouthrinses elmex® and Listerine® on plaque accumulation in 12-year-olds.

Material and methods: 30 12-year-old children took part in the clinical study. They were divided into three groups. Group I (10 people) was given Listerine® to home use. Group II (10 people) was given elmex® to home use. Group III (10 people) did not receive any mouthrinses. Following indices were used in first and base study Plaque Index (PI), Approximal Plaque Index (API) and Sulcus Bleeding Index (SBI). The statistical analysis was performed using T test for related samples and Spearman rank order correlations.

Results: Mean PI lessened in group I (Listerine®) from 0.996 to 0.804 and group II (elmex®) from 0.807 to 0.698. In group III it stayed almost at the same level. In all children values of API and SBI decreased after two weeks. Reduce of API in participants using Listerine® was important statistically and it lessened from 57.4% to 48.1% (reduction by 16.2%). The other results of API and SBI were not statistically important. API in children using elmex® lowered by 15.5%. Bleeding (SBI) in Listerine® group decreased by 21.5% and in elmex® group decreased by 24.5%. In control group diminish of SBI was only by 14.4%.

Conclusions: In summary, this study has demonstrated that additional rinsing helped in reducing plaque and gingivitis in 12-year-olds but it is not as essential as motivation to everyday oral hygiene.

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Key words: plaque, gingivitis, mouthrinses.

Introduction

Dental plaque is an essential etiological factor of caries and gingivitis. Nowadays dental plaque is regarded as microbial biofilm. Bacteria in biofilms are different from the same species freely floating in saliva. They develop phenotypes that can be more resistant to microbial agents [1,2]. There is a cause-consequence association between dental plaque and gingivitis. If young supragingival plaque is allowed to grow without any oral hygiene practice some changes will appear that result in gingivitis after 2-3 weeks [3,4]. Everyday oral hygiene meaning toothbrushing twice a day and cleaning interdental spaces with dental floss is an effective means of helping control dental caries and periodontal diseases. Mechanical home-care methods require time, manual dexterity and motivation. Even patients after professional oral hygiene training may miss hard-to-reach areas which are retention places of dental plaque. Especially uphill is daily interdental cleaning. Still slight per cent of patients uses dental floss in everyday routine. In countries where prophylaxis is well developed such as Canada 25% of population floss regularly, in England only 10 per cent [5]. That is why usage of chemotherapeutic agents can be an useful adjunct to mechanical methods. Mouthwashes are recommended after the patient has brushed and flossed his teeth. Market offers a lot of different mouthwashes. As an active ingredient they can comprise chlorhexidine, triclosan, fluorides, metal ions, oxidising agents, essential oils and many others. With the exception of 0.2% per cent chlorhexidine all mouthwashes are recommended as supplements to everyday oral hygiene. It is advised to test the products in the same way using long-term home use studies [2,6,7]. Investigator decided to test two of commercially available mouthrinses (elmex® and Listerine®) in a clinical study lasting two weeks.

Mouthrinse elmex® contains amine fluorides that are surface active. They concentrate easily on teeth surface and form

Table 1. **T test for related samples. No statistical differencies between PI 1 and PI 2 (p<0.05) in any group were noted**

	Variable	Mean	Std. Dv.	N	Diff.	Std. Dv. Diff.	t	df	p
group I Listerine®	PI 1	0.995833	0.349410						
	PI 2	0.804167	0.306218	10	0.191667	0.464894	1.303745	9	0.224680
group II elmex®	PI 1	0.807292	0.362515						
	PI 2	0.697917	0.406269	8	0.109375	0.502443	0.615710	7	0.557567
group III control	PI 1	0.712500	0.357055						
	PI 2	0.733333	0.337474	10	-0.020833	0.255533	-0.257817	9	0.802346

calcium fluoride areola that makes enamel more resistant to cariogenic bacteria. Supply of fluorine constantly released into saliva accelerate enamel remineralization. Additionally amine fluorides affect metabolism of bacteria creating dental plaque. It disturbs creating plaque biofilm [8].

Listerine® is a mouthwash that comprises essential oils (thymol, menthol, eucalyptol and methyl salicylate). It prevents dental plaque accumulation, effects on oral flora, has antimicrobial activity. It is effective against gingivitis and oral malodor [9].

As an examined group 12-year-old children were chosen. Interdental hygiene is especially important in adolescents 12-18 because of increase in caries on mesial and distal surfaces and arising gingivitis [10].

Material and methods

30 12-year-old children took part in the clinical study. They were divided into three groups. Group I (10 people) was given Listerine® to home use. Group II (10 people) was given elmex® to home use. Group III (10 people) did not receive any mouthrinses. Children belonging to group I and II were asked to use chemotherapeutic agents as the producer advises as a supplement to everyday oral hygiene, after every brushing.

Plaque Index (PI) (Silness i Löe) [11], Approximal Plaque Index (API) (Lange et al., 1977) [12] and Sulcus Bleeding Index (SBI) (Mühlemann and Son modified by Lange) [12,13] were recorded in screening examination. Dental plaque was stained with erythrosine tablets Red-Cote (manufactured by Butler). Each participant was instructed about oral hygiene.

Plaque Index (PI) (Silness i Löe) [11] was scored on four surfaces (that is buccal, lingual, mesial and distal) of six representative teeth (16, 12, 24, 44, 32, 36) after disclosing with erythrosine. Hygiene was assessed according to following scale:

- 0 – no plaque
- 1 – plaque invisible but can be found with periodontal probe at the gingival margin
- 2 – moderate plaque easily seen without probing
- 3 – ample plaque easily seen.

The mean index was calculated by dividing the sum of numbers from the scale by the total number of sites scored within the mouth.

Approximal Plaque Index (API) was scored after staining dental plaque. A periodontal probe was gently guided through approximal spaces of the first and third quadrants from the oral aspect and of the second and fourth quadrant from the buccal aspect. The plaque remnants were noted as "+" answer. Maximum 28 points were measured. Percent of surfaces with plaque was counted:

- API 100-70% bad oral hygiene
- API 70-40% average oral hygiene
- API 39-25% rather good oral hygiene
- API<25% optimum oral hygiene.

Sulcus Bleeding Index (SBI) was measured by guiding probe through the gingival sulcus in the first and third quadrants from the buccal aspect and in the second and fourth quadrant from the oral aspect. "+" or "-" answer was noted and per cent SBI was counted:

- SBI 100-50% heavy gingivitis
- SBI 50-20% moderate gingivitis
- SBI 20-10% light gingivitis
- SBI <10% clinically healthy gingiva.

After two weeks the indices were recorded again. Two subjects (group II) were exluded from the analysis because of missing the last examination.

The statistical analysis was performed using T test for related samples and. Spearman rank order correlations.

Results

Results are featured in *Tab. 1-4*. According to PI index in basic examination the best oral hygiene was noted in third group (0.713) after two weeks it stayed almost at the same level (0.733). Mean PI lessened in group I (Listerine®) from 0.996 to 0.804 and group II (elmex®) from 0.807 to 0.698. The differencies were not statistically important (*Tab. 1*).

In all children values of API and SBI decreased after two weeks. Reduce of API in participants using Listerine® was important statistically and it lessened from 57.4% to 48.1% (reduction by 16.2%). The other results of API and SBI were not statistically important. API in children using elmex® lowered by 15.5%. Bleeding (SBI) in Listerine® group decreased by 21.5% and in elmex® group decreased by 24.5%. In control group diminish of SBI was only by 14.4% (*Tab. 2*).

Tab. 3 displays oral hygiene according to Approximal Plaque Index. Most children had average oral hygiene (API 70-40%).

Correlation between API and SBI indicates that accumulation of dental plaque is associated with gingival bleeding (*Tab. 4*).

***Table 2*. Mean values of SBI% and API%**

	Index	Examination 1 ±SD	Examination 2 ±SD	Difference	Difference %	Statistical importance p<0.05
group I Listerine®	SBI%	26.5±6.4	20.8±6.9	5.7	21.5%	no
	API%	57.4±13.5	48.1±14	9.3	16.2%	yes
group II elmex®	SBI%	24.5±6.1	18.5±8.8	6	24.5%	no
	API%	55.625±15.9	47±22.1	8.625	15.5%	no
group III control	SBI%	22.2±11.8	19±11.6	3.2	14.4%	no
	API%	47.8±18.8	43.8±14.6	4	8.4%	no

***Table 3*. Oral hygiene of examined children according to API**

Examined group	Examination	API			
		100-70%	70-40%	39-25%	<25%
group I Listerine®	1	1 (10%)	8 (80%)	1 (10%)	0
	2	0	7 (70%)	2 (20%)	1(10%)
group II elmex®	1	1 (12.5%)	6 (75%)	1 (12.5%)	0
	2	2 (25%)	4 (50%)	1 (12.5%)	1 (12.5%)
group III control	1	1 (10%)	5 (50%)	3 (30%)	1 (10%)
	2	0	4 (40%)	6 (60%)	0

API 100-70% bad oral hygiene; API 70-40% average oral hygiene; API 39-25% rather good oral hygiene; API<25% optimum oral hygiene

***Table 4*. Spearman Rank Order Correlations**

	N	R	t(N-2)	p level
API% & SBI%	56	0.542660	4.747550	0.000016

Discussion

Conducted examination showed that using Listerine® mouthwash reduced amount of dental plaque and bleeding units in 12-year-olds. Reduction of interdental plaque according to API was statistically significant. Efficacy of Listerine® was acknowledged in many *in vitro* [14-17] and *in vivo* trials. References shows that essential oils are effective in people who suspend oral hygiene for a short time of examination [18] as well as in long-term observations when the mouthwash was used additionally to everyday oral hygiene [19-21].

Similar clinical trial with Listerine® lasting two weeks was undertaken by Skiba M. and collegues. Mouthwash was used in patiens with periodontis after professional scaling and root planning and oral hygiene instructions. Authors received reduction of API by 57.38% and SBI by 69.63%. Difference was statistically significant [22].

According to results elmex® is similarly effective to Listerine®. The lowest reduction of API and SBI was in control group. That indicates that by single oral hygiene instructions it is hard to motivate children to improve their oral hygiene. Śniatała R. noted that systematical brushing with repeated training, usage of fluoride toothpastes and dentist supervision are able to reduce the amount of dental plaque in children without additional aids [23]. Suchlike conclusions depicts as well Witt-Pawlowski K. who received statistically important reduction of API and SBI in 10--12-year-old children by 5 visit individual profilaxis that relayed on instruction, remotivation, fluorization, caries treatment and professional scaling [24].

In summary, this study has demonstrated that additional rinsing helped in reducing plaque and gingivitis in 12-year-olds but it is not as essential as motivation to everyday oral hygiene.

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Occurrence rate of oral *Candida albicans* in denture wearer patients

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Abstract

Purpose: The aim was to determine the fungi occurrence rate in the oral cavity of denture wearer patients in comparison to those without dentures.

Material and methods: The examinations were conducted in patients treated in two clinical departments of the University Hospital. Demographic data and those connected with basic diseases were collected and the evaluation concerning dentition and oral hygiene was performed. Samples for mycological examinations from the tongue dorsa, palatal mucosa, and mucosal surfaces of dentures were collected from patients with dentures while tongue and palate swabs were taken from those without dentures. For culture and identify of fungi standard methods were used.

Results: Dental and mycological examinations were performed in 95 patients, out of which 57 (60.0%) used complete or partial dentures and 38 (40.0%) had their own dentition (without dentures). Oral cavity revealed only growth of *Candida albicans* species, more frequently in patients with dentures (38/57; 66.7%) than in those without dentures (11/38; 28.9%) ($p=0.0003$). *C. albicans* statistically significantly more frequently was isolated in denture wearer patients with diabetes mellitus ($p=0.0207$) and without diabetes ($p=0.0376$) comparing to such groups of patients but without dentures. Among 32 patients with diabetes mellitus, 14 (43.8%) revealed *C. albicans*; this rate was comparable with 9/23 (39.1%) patients without diabetes ($p>0.05$). A similar analysis, conducted in 25 surgical patients with abdominal cancer and 15 – without – cancers, did not show statistically significant differences in the incidence rate of *C. albicans*; it also concerned denture wearers (14/16; 87.5%) and non-wearing dentures (5/9; 55.6%) ($p>0.05$) with cancer. In 37 (64.9%) wearer patients denture stomatitis was observed, associated mainly with *C. albicans* infections (29/37; 78.4%).

Conclusions: 1) Mycological findings from the present study do not indicate that diabetes mellitus or advanced cancer has a significant effect on oral colonisation by *Candida albicans* or other species of *Candida* genus. 2) The occurrence rate of oral *Candida albicans* in patients with dentures (diabetic and non-diabetic, cancer and non-cancer patients) was higher than in patients without dentures ($p<0.05$).

Key words: oral *Candida albicans*, denture plaque, denture wearers, diabetes mellitus, denture stomatitis, oral hygiene.

Introduction

Candida is present in the oral cavity of almost half of the population and has been shown be prevalent in people with diabetes mellitus as well [1-4]. Studies have shown a higher prevalence of *Candida* in diabetic versus non-diabetic individuals [1,3]. In addition, significantly higher prevalence of *Candida* infection in people with diabetes was reported [2]. *Candida* infection is also found commonly in denture wearers [5]. According to a survey of the literature on oral yeast (*Candida albicans* and other *Candida* species) isolations from subjects without signs of mucosal diseases the median carriage rate was 34.4% among healthy adults whereas it was 54.7% in hospitalized patients [6].

Candida infection (candidiosis or candidiasis) can occur as a side effect of medications such as broad-spectrum antibiotics, antihistamines, chemotherapy or radiotherapy. Other disorders associated with development of xerostomia include diabetes, drug abuse, malnutrition, immune deficiencies, and old age [4,7-9].

The manifestation of oral candidiosis (candidiasis) can occur in many different forms and include median rhomboid

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Table 1. **Demographic characteristics and risk factors for oral *Candida albicans* in hospitalized patients**

	Total	Denture wearers (n=57)	No-denture wearing (n=38)	p-value
Demographic data				
Age (years)				
Mean ±s.d.		66.4±11.7	49.9±16.0	0.0000**
Range		40-83	19-76	
Number of patients	95/49*	57/38	38/11	0.0003
≤44 years	14/3	1/0	13/3	n.s.
45-54 years	23/10	12/7	11/3	n.s.
55-74 years	43/28	31/23	12/5	n.s.
≥75 years	15/8	13/8	2/0	n.s.
Gender				
Male	42/23	23/17	19/6	0.015
Female	53/26	34/21	19/5	0.0286
Oral status				
Oral hygiene status				
– good	29/10	16/7	13/3	n.s.
– fair	54/33	35/27	19/6	0.0028
– poor	2/8	6/5	6/3	n.s.
Tongue disorders	37/18	23/12	14/6	n.s.
Xerostomia	24/18	15/12	9/6	n.s.
Denture stomatitis		37/29	-	
Diabetic patients	32/14	19/12	13/2	0.0207
Non-diabetic patients	23/9	16/9	7/0	0.0376
Cancer patients	25/19	16/14	9/5	n.s.
Non-cancer patients	15/7	6/3	9/4	n.s.

* No. of patients/No. of *C. albicans*; ** comparison by Student's t-test

glossitis, atrophic glossitis, denture stomatitis (stomatitis prothetica), and angular cheilitis [4,10]. Usually, oral candidiosis is associated with a high density of yeasts in the lesions [8,11]. Oral candidiosis have been reported in 9% to 65% of the population [7,12-14]. These variations are far too important to be explained by demographic variations or socio-economic dissimilarities alone, but may be linked, in part, to differences in denture usage and hygiene habits as well as to underlying systemic predisposing factors [9,11].

Our previous studies have shown a high incidence of isolation of *Candida* spp. from oral cavities of patients with denture stomatitis (94%), healthy denture weares (75%) and healthy people with their own dentition (41%) [15]. The aim of the present study was to assess the prevalence of yeast in the oral cavities of denture wearers and without denture patients with diabetes mellitus or abdominal cancer patients.

Material and methods

A total of 95 patients were evaluated in this study: 55 patients (32 with diabetes mellitus) admitted to the Department of Endocrinology, Diabetology and Internal Medicine and 40 patients treated in the II Department of General Surgery and Gastroenterology (25 with abdominal cancer). Each patients completed a medical and dental history and signed an informed consent document. All patients accepted an oral examinations. The patients were divided into two groups, according to the presence or absence of dental prosthesis (denture).

Samples were obtained by swabbing the oral mucosa (palatal mucosa and tongue dorsa) of all patients and the contiguous denture surfaces of patients with dental prosthesis. All oral specimens were placed on Sabouraud glucose agar. All isolated yeasts were identified with classic methods and carbohydrate assimilation patterns using commercial kit API 20C AUX (bioMérieux, ATB Expression) as a previous described [16].

The study protocol was approved by the Local Bioethics Committee of the Medical University of Białystok.

Statistical analysis

Student's t-test was used to analyse the differences between the means (shown as mean ±s.d.). The Chi-squared test was used to analyse the differences between the frequencies in groups. Groups or subgroups were considered significantly different from each other if $P<0.05$. All statistical calculations were performed using Statistica 6.0 for Windows.

Results

The prospective studies were performed in 95 patients treated in two in patient departments, out of which, 57 (60.0%) used complete or partial dentures and 38 (40.0%) had their own dentition (patients without denture) (*Tab. 1*).

***Table 2*. Prevalence of oral *Candida albicans* and denture stomatitis in denture wearer patients**

	Denture stomatitis		Newton types of stomatitis		
	No (n=20)	Yes (n=37)	I (n=25)	II (n=9)	III (n=3)
Demographic data					
Age (years)					
Mean ±s.d.	63.1±11.69	67.51±11.13	62.5±10.77	64.2±12.29	78.3±8.1
Range	44-80	40-83	40-79	50-83	69-83
Number of patients	20/9*	37/29	25/19	9/8	3/2
Gender					
Male	6/3	17/14	11/8	4/4	2/2
Female	14/6	20/15	14/11	5/4	1/0
Oral status					
Oral hygiene status					
– good	16/7	0	0	0	0
– fair	4/3	31/24	24/18	5/5	2/1
– poor	0	6/5	1/1	4/3	1/1
Tongue disorders	7/1	16/11	13/8	1/1	2/2
Xerostomia	1/1	14/11	11/8	2/2	1/1
Diabetic patients	5/2	14/10	8/5	3/3	3/2
Non-diabetic patients	8/3	8/6	6/5	2/1	0
Cancer patients	4/3	12/11	9/8	3/3	0
Non-cancer patients	3/1	3/2	2/1	1/1	0

* No. of patients/No. of *C. albicans*

A total of 49 (51.6%) candidial strains from examined patients was isolated. In 35/49 (71.4%) patients *Candida albicans* strains were recovered from both in palatal mucosa and tongue dorsa. Oral cavity revealed only yeasts of *Candida albicans* species, more frequently in patients with dentures (38/57; 66.7%) than in those without dentures (11/38; 28.9%) (p=0.0003). *Candida albicans* statistically significantly more frequently was isolated in denture wearer patients with diabetes mellitus (p=0.0207) and without diabetes (p=0.0376) comparing to such groups of patients but without dentures (*Tab. 1*). Among 32 patients with diabetes mellitus (27 type 2 and 5 type 1), 14 (43.8%) revealed *C. albicans*; this rate was comparable with 9/23 (39.1%) patients without diabetes (p>0.05). A similar analysis, conducted in 25 surgical patients with abdominal cancer, and 15 non-cancer patients, did not show statistically significant differences in the incidence rate of *C. albicans*; it also concerned wearing dentures with cancer (14/16; 87.5%) and without dentures with cancer (5/9; 55.6%) (p>0.05) (*Tab. 1*).

Among putative risk factors evaluated, wearing denture patients (see above) and only older age (55-74 years: 28/43; 65.1% vs <44 years old: 3/14; 21.4%) (p=0.01) were associated with the most frequent isolated *C. albicans* (*Tab. 1*).

We also demonstrated that the presence of *C. albicans* was more frequent in denture-related stomatitis (29/37; 64.9% +ve vs 9/20; 45.0% -ve) (p=0.0107) (*Tab. 2*). The presence of denture-related stomatitis was assessed according to a modified version of Newton's classification [17]. The severity of the palatal inflammation was classified as: 1) no stomatitis, no evidence of palatal inflammation; 2) stomatitis Newton type I, petechiae dispersed throughout all or any part of palatal mucosa in contact with the denture; 3) Newton type II, macular erythema without hyperplasia; or 4) Newton type III, diffuse or generalized erythema with papillary hyperplasia.

Statistical analysis showed that the frequency and the category of stomatitis between the risk factors was not significantly different (p>0.05), except for tongue disorders. Frequency of *C. albicans* present in patients with stomatitis and tongue disorders (mainly atrophic tongue) was significantly higher (11/16; 68.8%) than in free-stomatitis patients with tongue disorders (1/7; 14.3%) (p=0.05) (*Tab. 2*). Statistical analysis according to Newton types had shown no significant relation between stomatitis and number of detected yeast colonies (density as CFU) on dentures (denture plaque) and palatal mucosa and/or tongue dorsa (data not shown).

Discussion

Candidal infections are a major problem in the world, especially among the immunosuppressed people [2,4,7-9,11,13]. Furthermore, increased susceptibility to oral infections with *Candida* spp. has long been associated with diabetes mellitus [1-4,18-21], but the results remain controversial and contradictory [18-21]. In the present study 14/32 (44.8%) diabetic patients were found to carry *Candida albicans* in their oral cavity. This finding showed no statistical significance when compared with non-diabetic patients (9/23; 39.1%) (p=0.7319). A similar trend was observed by Kadir et al. [21] and by Sahin et al. [20].

Some investigators have demonstrated that candidal carriage is higher among diabetics wearing dentures [5,12,14,17]. In our study the frequency of oral candidal isolates was more common in 12/19 (63.2%) isolates in diabetic patients with denture

compared with 2/13 (15.4%) isolates in diabetic patients without denture (p=0.0207), but insignificantly related in age and sex distribution of both groups (p>0.05). Only *Candida albicans* was isolated in both diabetic groups.

Candida colonization in denture wearers, especially immunocompromised patients, can be disruptive to dental treatment and may be a barrier to patient health. The surface irregularities of acrylic resin are a factor in the entrapment of microorganisms, especially *Candida albicans*. Consequently, controlling the spread of fungal infection in risk patients who wear removable prostheses and who are more susceptible to fungal infections because of their immunosuppression is of critical importance. It is important in denture wearers with HIV infections [22] and diabetic patients [5,12,14,17-20,23,24] or advanced carcinoma [23,24].

Oral fungal infections frequently develop in individuals with advanced cancer, especially in the patients receiving palliative care for advanced malignant disease. Bagg et al. [23] showed that patients with advanced cancer have demonstrated a high incidence (51%) of oral colonization with non-*C. albicans* yeasts, many of which had reduced susceptibility to fluconazole and intraconazole (e.g. 72% resistant *C. glabrata*).

The oral yeast carriage in 66% of patients with advanced cancer by Davies et al. [24] were observed. The frequency of isolation of individual species was: *Candida albicans*, 46%; *C. glabrata*, 18%; *C. dubliniensis*, 5%; others, <5%. Oral yeast carriage was associated with denture wearing (p=0.006) [24]. In our study also a high isolation frequency (76% or 87.5% for denture wearers) was observed, but only one species, *Candida albicans*, from patients with abdominal cancer.

Our results also show that in denture-related stomatitis (denture stomatitis), the presence (colonization/infection) of *Candida albicans* on the denture is probably linked to extensive inflammation. In addition, the isolated *Candida* spp. associated with dentures are related to the poor hygienic condition of the prostheses, to the long time of the usage, wearing dentures at night and to the modifications of the hard supporting tissues [17,19]. In contrast, denture stomatitis was observed in healthy and diabetic or advanced cancer groups with almost the same frequency [15,17,19,23,24].

Whether *C. albicans*, alone or with other organisms, is involved in onset or development of denture related stomatitis remain to be determined.

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Aerobic and anaerobic bacteria in subgingival and supragingival plaques of adult patients with periodontal disease

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Abstract

Purpose: Clinical, epidemiological and microbiological examinations of adult patients with periodontal disease.

Material and methods: The study of population consisted of 21 subjects (13 female and 8 male) aged 38-58 years, treated in the Outpatient Department of Periodontology. Dental examinations were performed at an artificial light and using a WHO periodontometer, a mirror and a probe. Periodontal status was assessed by determination of the probing pocket depth (CPI), gingival state (GSBI according to Mühlemann and Son), and oral hygiene index (according to Silness and Löe). Material for microbiological examination was collected from subgingival and supragingival plaques of each patient. Additionally, pus was obtained from 8 patients and periodontal pocket fluid from 2 patients. The samples were examined for the presence of aerobic and anaerobic bacteria and *Candida* yeasts. Standard procedures were used for culture and identification of bacteria and fungi.

Results: *Candida* yeasts were not isolated from adults with periodontal disease. In 19/21 patients, cultures of both aerobic and anaerobic bacteria from subgingival and supragingival plaque samples were positive. A total of 42 bacterial strains were isolated from subgingival plaques, of which 24 (57.1%) belonged to 7 anaerobic species and 18 (42.9%) to 12 aerobic species ($p>0.05$). There were more aerobic (33/53; 62.3%) than anaerobic bacteria (20/53; 37.7%) ($p<0.05$) in supragingival plaques. Anaerobes were isolated more frequently than aerobes from the abscess ($p<0.05$).

Conclusions: 1) In adult patients with periodontal disease, Gram-positive anaerobes, including *Peptostreptococcus*, were the predominant bacteria in the subgingival plaque.

2) While in the supragingival plaque, Gram-positive aerobic cocci (*Streptococcus* and *Staphylococcus*) were predominant.

Key words: adult periodontitis, supra- and subgingival plaques, bacterial composition, anaerobic bacteria.

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Introduction

Periodontal diseases defines a broad group of diseases affecting the periodontal tissue, the most common are inflammatory processes of the gingiva and tissues attaching to the tooth [1]. These diseases are usually associated with microbial infection due to accumulation of a plaque biofilm and calculus.

Periodontitis refers to a group of more advanced and related diseases within the broad heading of periodontal disease. It can be defined as "an apical extension of gingival inflammation to involve the tissues supporting the tooth, including periodontal ligament and bone" [1]. The destruction of the fibre attachment results in a periodontal pocket. This wide spectrum of diseases has recently been reclassified by Armitage [2], and at least 48 specific periodontitis categories are now recognized. By far the most common is chronic periodontitis and this is the major cause of tooth loss in the adult population. The disease is mediated by the microflora forming the plaque biofilm on the tooth surface. Additionally, as a consequence of the immune response elicited by the bacteria further destruction may occur due to the host inflammatory response.

Chronic periodontitis in adults patients results from a complex interplay of the mixed polymicrobial infection and host response. The adherent microbes evoke release of a number of inflammatory mediators in the underlying soft tissue. In fact, these activation products ultimately result in the destruction of host tissue [1-3].

Human periodontitis is associated with a widely diverse

and complex subgingival microbiota encompassing both Gram-positive and Gram-negative bacteria, facultative and anaerobic organisms, and possibly yeasts. At least nearly 500 bacterial strains have been recovered from the subgingival crevice, a particularly well-studied microbial niche [4-6]. Most of these strains are thought to be commensals and a smaller number, potential opportunistic pathogens.

In the oral cavity, yeasts commonly colonize the tongue, palate, and buccal mucosa and may occur in subgingival plaque of adults with severe periodontitis [7,8]. Yeasts, especially *C. albicans*, have been recovered from periodontal pockets in a large number (>15%) [5,7-10]. In addition to periodontal diseases, oral yeast have been related with enamel and root caries [7,8,11].

Clinical, epidemiological and microbiological examinations of adult patients with periodontal disease are the purpose of our own studies.

Material and methods

The study of population consisted of 21 subjects (13 female and 8 male) aged 38-58 years, treated in the Outpatient Department of Periodontology. Each subject completed a medical and dental history and signed an informed consent document.

Dental examinations were performed at an artificial light and using a WHO periodontometer, a mirror and a probe. Periodontal status was assessed by determination of the probing pocket depth (CPI), gingival state (GSBI according to Mühlemann and Son) and oral hygiene index (according to Silness and Löe) [12]. All teeth were examined at six sites and periodontal pockets over 3.5 mm were recorded. The Community Periodontal Index (CPI) [13], presence of suppuration and bleeding on probing were recorded before the periodontal treatment.

Material for microbiological examinations was collected from supragingival and subgingival plaques of each patients. Subgingival plaque samples taken by curettage from deep suppurating periodontal pockets. Additionally, pus was obtained from 8 patients, and periodontal pocket fluid from 2 patients. The samples were transported in transport medium to the Department of Microbiology, Medical University of Białystok and were cultured routinely aerobically and anaerobically on selective and non-selective agars for a various groups of bacteria. Sabouraud glucose agar was used for culture *Candida* spp. Bacterial and fungal identification was based on the colony morphology and pigmentation, staining and biochemical reactions (API commercial kits; bioMérieux) [14-16].

The study was approved by Ethical Committee of the Medical University of Białystok. Statistical analysis was performed using chi-squared test. In this analysis, $p \leq 0.05$ was considered as statistically significant.

Results

Dental examination carried out in 21 subjects treated in the Outpatient Department of Periodontology. The result of examination registered according to WHO Oral Health Assessment Form (1986). The Community Periodontal Index (CPI) was recorded in all of the patients. They had CPI scores of 4 in at least 1 sextant. According to CPI score 0-4 scale in the examined teeth we observed the following: 0-4 sextants, 1-7, 2-11, 3-55, and 4-49 sextants. In the patients with periodontal disease 164 pockets, with depth ranged between 3.5 to 5.5 mm were found; 56 pocket were more than 5 mm deep. Gingival state was assessed according to Gingival Sulcus Bleeding Index (GSBI), periodontal pocket bleeding according to Mühlemann and Son. We record code 4 in 10 (47.6%) patients, code 5 in 9 (42.9%) patients, code 2 and 3 in one patients (both with 4.8%).

Material for microbiological examinations was collected from supragingival and subgingival plaques of each patients. The results of this study are presented in *Tab. 1*. No growth of bacteria and fungi seen in samples obtained from two patients. Moreover, no fungi isolated from the remained patients. Aerobic bacteria isolated more frequently from supragingival plaque

Table 1. **Number of bacterial strains isolated from supra- and subgingival plaques and gingival abscess in adult patients with periodontitis**

Bacterial species	Supragingival plaque (n=19)	Subgingival plaque (n=19)	Gingival abscess (n=8)
I. Aerobic bacteria	**33**	**18**	**5**
1. Gram-positive	21	11	3
Gram-positive cocci	21	11	3
Streptococcus spp.	11	7	2
Gemella morbillorum	4	1	0
Staphylococcus spp.	5	3	1
S. aureus	1	0	0
Coagulase-negative	4	3	1
Micrococcus spp.	1	0	0
2. Gram-negative	12	7	2
Gram-negative cocci	10	6	2
Neisseria spp.	10	6	2
Gram-negative rods	2	1	0
Haemophilus parainfluenzae	2	0	0
Escherichia coli	0	1	0
II. Anaerobic bacteria	**20**	**24**	**15**
1. Gram-positive	14	14	14
Gram-positive cocci	10	9	5
Peptostreptococcus spp.	10	9	5
Gram-positive rods	4	5	9
Bifidobacterium spp.	1	1	2
Eubacterium aerofaciens	0	0	1
Lactobacillus fermentum	2	2	3
Propionibacterium spp.	0	1	0
Actinomyces naeslundii	1	1	3
2. Gram-negative	6	10	1
Gram-negative rods	1	2	0
Prevotella oralis	0	2	0
Bacteroides ovatus	1	0	0
Gram-negative cocci	5	8	1
Veillonella parvula	5	8	1
Bacteria:	**53**	**42**	**20**
Gram-positive	35	25	17
Gram-negative	18	17	3

***Table 2*. Isolation frequency (%) of aerobic and anaerobic bacteria in adult periodontitis according to oral hygiene status**

Bacterial species	Supragingival plaque (19)			Subgingival plaque (19)			Gingival abscess (8)		
	*1(5)	2(9)	3(5)	1(5)	2(9)	3 (5)	1(2)	2(1)	3(5)
I. Aerobic bacteria									
1. Gram-positive									
Gram-positive cocci									
Streptococcus spp.	60.0	66.7	40.0	20.0	44.4	40.0	0	0	40.0
Gemella morbillorum	40.0	11.1	20.0	0	0	20.0	0	0	0
Staphylococcus spp.	40.0	33.3	0	20.0	22.2	0	50.0	0	0
S. aureus	20.0	0	0	0	0	0	0	0	0
Coagulase-negative	20.0	33.3	0	20.0	22.2	0	50.0	0	0
Micrococcus spp.	0	11.1	0	0	0	0	0	0	0
2. Gram-negative									
Gram-negative cocci									
Neisseria spp.	40.0	44.4	80.0	0	44.4	40.0	50.0	0	20.0
Gram-negative rods									
Haemophilus parainfluenzae	0	11.1	20.0	0	0	0	0	0	0
Escherichia coli	0	0	0	0	11.1	0	0	0	0
II. Anaerobic bacteria									
1. Gram-positive									
Gram-positive cocci									
Peptostreptococcus spp.	60.0	44.4	60.0	20.0	44.4	80.0	100.0	100.0	40.0
Gram-positive rods									
Bifidobacterium spp.	0	11.1	0	0	0	20.0	0	100.0	20.0
Eubacterium aerofaciens	0	0	0	0	0	0	50.0	0	0
Lactobacillus fermentum	40.0	0	0	0	22.2	0	0	0	60.0
Propionibacterium spp.	0	0	0	0	11.1	0	0	0	0
Actinomyces naeslundii	0	11.1	0	0	11.1	0	0	0	80.0
2. Gram-negative									
Gram-negative rods									
Prevotella oralis	0	0	0	0	22.2	0	0	0	0
Bacteroides ovatus	20.0	0	0	0	0	0	0	0	0
Gram-negative cocci									
Veillonella parvula	40.0	11.1	40.0	40.0	33.3	60.0	0	0	20.0

*Oral hygiene status: 1 – good, 2 – fair, 3 – poor } (No. of patients)

of 19/21 patients (33/53; 62.3%) compared to anaerobic bacteria (20/53; 37.7%) (p=0.011). Moreover, there were more isolation of Gram-positive bacteria (aerobic and anaerobic) than Gram-negative bacteria (aerobic and anaerobic) (p=0.0019). A total of 42 bacterial strains were isolated from subgingival plaques of the same patients, of which 24 (57.1%) belonged to 7 anaerobic species and 18 (42.9%) to 12 aerobic species (p>0.05) (*Tab. 1*). No significant difference observed in the isolation frequency between anaerobic and aerobic bacteria (p=0.19) from subgingival plaques.

Additionally, a total of 20 bacterial strains were isolated from the abscess obtained from 8 patients, where anaerobic (75%) were isolated more frequently than aerobic (25%) (p=0.0015). Gram-positive (85%) bacteria more frequent were isolated than Gram-negative bacteria (15%) (p=0.0000) (*Tab. 1*).

Five strains of bacteria were isolated from the periodontal pocket fluid of 2 patients in which 2 were anaerobic (*Veillonella* spp. and *Peptostreptococcus* spp.) and 3 were aerobic (*Neisseria mucosa*, *Streptococcus constellatus* and *S. vestibularis*).

The isolation rate of bacteria in patients with periodontitis in relation to oral hygiene status shown in *Tab. 2*. Oral hygiene status assessed by Plaque Index (PI) (according to Silness and Löe) in 3 steps scale [12]. PLI=1 (hygiene good) seen in 5 (26.3%) patients, PLI=2 (hygiene fair) in 9 (47.4%), PLI=3 (hygiene poor) in 5 (26.3%) patients. A significant difference observed in the frequency of isolation of anaerobic Gram-positive bacteria from supragingival plaques in patients with fair and poor oral hygiene (8/19; 42.%) compared with subject with good oral hygiene (1/19; 5.3%) (p=0.0076). Anaerobic Gram-positive bacteria were isolated more frequently than aerobic bacteria from the gingival abscess (*Tab. 2*).

Discussion

The oral cavity is characterized by harbouring indigenous microbiota. The ability of microorganisms to colonize the different oral surfaces depends mainly on their binding potential.

Various environmental factors and host factors are involved in the harbouring of microorganisms and microbial composition. Many indigenous microbiota are anaerobes and these microorganisms can be associated with oral infections and be the origin of distant infection [1,2,14,16-22]. The most frequent oral anaerobic infections include gingivoperiodontal diseases, pulpal and periapical infections, peri-implantitis and pericoronarities [17].

Gingivoperiodontal diseases, including gingivitis and periodontitis, are caused by dental plaque, which is a biofilm [1,18,23,24]. It has been observed that 1g of dental plaque contains more that 10^{11} microorganisms [18].

The biofilm present in the gingival crevice, and later in the periodontal pocket, is extremely diverse, with up to 100 culturable species from a single pocket [20]. Since such a diverse flora is present, trying to identify the particular species responsible for disease initiation and progression is a very complex, and difficult undertaking. Therefore, it is to be expected that a progressively more diverse and anaerobic flora will be isolated during disease progression. Moore and Moore [25] presented large numbers of anaerobes increase in their overall proportions during disease progression and, conversely, aerobe and facultative species, decrease (changes in the microflora of the biofilm as a function of periodontitis severity).

The World Workshop on Clinical Periodontology (American Academy of Periodontology Consensus report 1996) has designated three species as aetiologic agents of periodontitis in susceptible hosts, namely *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* (*T. forsythensis*; formerly *Bacteroides forsythus*) [1].

Socransky et al. [26] were found five major complexes associated with different species of microorganisms in periodontal disease. The "black" or "red" complex consisting of *Tannerella forsythia*, *Porphyromonas gingivalis* and *Treponema denticola* appears to relate closely to clinical measures of periodontal disease, such a bleeding on probing and increasing pocket depth.

A recent comprehensive study using PCR and sequence analysis 16S rRNA from bacteria in subgingival plaque suggests that approximately 415 species are likely to be present [27].

Several bacterial species or cluster of species have been implicated in the aetiology of periodontitis [20,26,27]. Adult periodontitis is associated with a group of bacteria and different complexes have been described from subgingival plaque samples [17,23,26].

Although extensive microbial analyses have been performed from subgingival plaque samples of periodontitis patients, systematic analysis of subgingival microbiota has not been carried out in north-region of Polish population so far. Purpose of this study was to describe the prevalence of bacterial composition in our patients by culture methods. The present study demonstrated a different microbiota between supra- and subgingival plaque and gingival abscesses. The putative periodontal pathogens, such as *Porphyromonas gingivalis*, *Prevotella intermedia*/*P. nigrescens* and *Actinobacillus actinomycetemcomitans* were not isolated by us. Leonhardt et al. [28] these periopathogens were found in 60% subgingival samples. In our study, we isolated only 2 strains of *Prevotella oralis* from subgingival plaque and 1 strain of *Bacteroides ovatus* from supragingival plaque. *Veillonella parvula* strains were more frequent isolated from subgingival plaque (8/19; 42.1%) than supragingival plaque (5/19; 26.1%), but no statistical difference was observed (p=0.305).

Kamma et al. [29] have examined the microflora of severe, moderate and minimal lesions in young adults with rapidly progressing periodontitis, and have observed microbial complexes associated with severe and moderate lesions, while in small lesions species of *Actinomyces* and *Streptococcus*, *Capnocytophaga ochracea*, *Haemophilus segnis* and V*eillonella parvula* were identified. *Veillonella* species, *Fusobacterium*, *T. denticola* and *P. gingivalis* have all been associated with gingivoperiodontal infection and with halitosis [17].

It is, however, interesting to note that Gram-positive anaerobic bacteria, especially *Peptostreptococcus* spp., were isolated with high rates in both supragingival plaque (10/19; 52.6%) and subgingival plaque (9/19; 47.4%) (p=0.7456). Some species of *Peptostreptococcus* belonged to a second major group (complex) according to Socransky et al. [26]. The second group microorganisms include *Fusobacterium nucleatum*, *Campylobacter rectum*, *Eikenella corrodens*, *Eubacterium nodatum*, *Selenomonas noxia*, *Peptostreptococcus micros*, *Streptococcus intermedius* and *Treponema denticola* [1,4,17,20,25,26]. The role of these organisms in adult periodontitis initiation and/or progression is much less defined than that of the "black" complexes. The significance of other complexes is also not yet understood.

None of the patients with periodontitis were found to harbour *Candida* spp. Other authors also infrequently seen yeasts in association with periodontitis [7,9,10,28]. However, Järvensivu et al. [8] suggested that *C. albicans* could have a role in the infrastructure of periodontal microbial plaque and in its adherence to the periodontal tissues. This authors results also indicate that hyphal germination starts in the gingival pocket while fungal elements could not be seen in the epithelium [8].

Conclusions

In conclusion, the microbial diversity found in the present study, should therefore be considered in the treatment strategy of periodontitis in adult patients. Microbiological studies of periodontitis patients from different geographic regions including developed and developing countries, indicate variations in the numbers and species of cultivable bacteria [3-9,17-20,23-27,30].

The association of oral infections with infective endocarditis has been demonstrated [17,18,23]. Recently, periodontal disease has been regarded as a high-risk factor for coronary diseases, arteriosclerosis, myocardial infarction, pneumonia, pre-term births and low birth weight [11,18-23]. Hence, the clinical and microbiological study of dentate subjects are mandatory. There is a variety of microbiological diagnostic methods for oral infections, the therapy of which depends on a lose relation between the clinician and the microbiologist. Dentistry should assume this new challenge of team work interaction in order to prevent and solve oral and systemic infections.

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Aerobic bacteria in the oral cavity of patients with removable dentures

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Abstract

Purpose: Determination of bacterial composition in the oral cavity of patients with removable dentures and with own dentition (without dentures).

Material and methods: Bacteriological investigations were performed in 55 patients from the department of internal medicine (32 diabetic patients) and 40 patients treated in surgical department (25 patients with malignancy). Palate mucosa and tongue dorsa swabs were collected from two groups of patients, and additionally swabs from mucosal part of denture surfaces in prosthetic patients. Cultures in oxygenic and microaerophilic (5% CO_2) conditions were conducted on solid non-selective and selective media as well as media enriched with 5% sheep blood. Standard procedures of bacterial culture and identification were applied.

Results: Among 95 of examined patients, 57 (60.0%) with removable dentures and 38 (40.0%) had their own dentition. As far as prosthetic patients were concerned, the rate of bacterial isolations from palate, tongue dorsa and denture plaque swabs were generally comparable ($p>0.05$); in number and species compositions. Statistically significant differences were observed in the bacterial composition of denture plaques, palate and tongue dorsa in patients with and without abdominal cancers. Patients without cancer did not reveal staphylococci and enteric bacteria in the samples from a various sites of their oral cavities. These bacteria were most common in cancer patients. Similar (in number and species) composition of bacteria occurred in palate and tongue swabs in patients without dentures ($p>0.05$). The incidence rate of aerobic bacteria in denture plaques and palatal mucosa of patients with (37/57; 64.9%) and without (20/57; 35.1%) denture associated stomatitis were comparable (except for *Neisseria* spp.).

Conclusions: 1) Generally, there were no statistically significant differences in species composition of bacteria isolated from the hard palate and tongue dorsa in patients with and without removable dentures. 2) *Staphylococcus* spp. and Gram-negative enteric bacilli were isolated more often from denture plaque, palate and tongue dorsa of cancer patients than from patients without cancer ($p<0.05$). 3) *Staphylococcus* spp. was isolated more frequently from denture plaques of diabetic patients compared with non-diabetic patients ($p<0.05$). 4) No significant differences observed in isolation frequencies (%) of aerobic bacteria in denture plaques and palatal mucosa of patients with and without denture associated stomatitis.

Key words: bacterial composition, denture plaque, diabetic patients, patients with malignancy, patients with removable dentures, non-denture patients, denture associated stomatitis.

Introduction

Wearing removable dental prosthesis causes an alteration in the oral microflora [1]. For certain individuals, this new environment is responsible for the development of a particular condition: dental prosthetic, stomatitis or denture associated stomatitis.

Denture stomatitis is characterized by mucosal inflammation and redness underneath a denture [2]. It is caused by the microbial biofilm on the fitting surface of the denture rather than on the mucosal surface of, for example, the palate [3].

Denture associated stomatitis (DAS) or chronic atrophic candidosis is one of the most common clinical presentations of oral candidosis [4], affecting 24-60% of otherwise well denture wearers [5]. Nearly 90% of cases are thought to be caused by

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Table 1. **Isolation frequency (%) of bacteria in hard palate and tongue dorsa of denture wearers and non-denture wearing patients**

Bacterial species	Isolation frequency (%)			
	Denture (n=57)		**No-denture** (n=38)	
	Palate	Tongue	Palate	Tongue
Gram-positive bacteria				
Gram-positive cocci				
Staphylococcus spp.	21.1	22.8	21.1	34.2
S. aureus	8.8	8.8	0	5.3
Coagulase-negative	12.3	14.0	21.1	28.9
Streptococcus spp.	93.1	91.2	97.4	89.5
S. mitis	15.8	19.3	23.7	26.3
S. sanguis	15.8	22.8	21.1	13.2
S. oralis	8.8	8.8	7.9	10.5
S. salivarius	24.6	21.1	23.7	13.2
S. vestibularis	26.3	14.0	18.4	10.5
S. anginosus	1.8	1.8	2.6	5.3
S. intermedius	0	3.5	0	10.5
Gram-negative bacteria				
Gram-negative cocci				
Neisseria spp.	71.9	73.7	65.8	78.9
N. mucosa	8.8	12.3	13.2	7.9
N. sicca	14.0	17.5	13.2	18.4
N. subflava	28.1	26.3	23.7	34.2
N. flavescens	21.1	17.5	15.8	18.4
Gram-negative rods				
Haemophilus parainfluenzae	10.5	26.3	13.2	13.2
Enterobacteriaceae spp.	17.5	12.3	5.3	10.5
Escherichia coli	3.5	1.8	2.6	2.6
Klebsiella pneumoniae	8.8	7.0	2.6	5.3
Morganella morganii	0	0	0	2.6
Enterobacter cloacae	3.5	1.8	0	0
Citrobacter freundii	1.8	1.8	0	0
Pseudomonas aeruginosa	8.8	7.0	2.6	5.3
Acinetobacter spp.	0	0	2.6	2.6

yeasts [3,6], typically *Candida albicans*, although lesions have also been associated with variety of other *Candida* spp. [4-7] as well as bacteria from several genera [2,4,8,9]. Hence, the aim of the present study was to determine the bacterial composition in the oral cavity of patients with removable dentures and with own dentition (without dentures).

Material and methods

Bacteriological investigations were performed in 55 patients (24 male and 31 female) from the Department of Endocrinology, Diabetology and Internal Medicine (32 diabetic patients) and 40 patients (18 male and 22 female) treated in the II Department of General Surgery and Gastroenterology (25 abdominal cancer patients). Palate mucosa and tongue dorsa swabs and additionally swabs of mucosal part of denture surfaces in prosthetic patients were collected. Cultures in oxygenic and microaerophilic (5% CO_2) conditions were conducted on agar non-selective and selective media as well as media enriched with 5% sheep blood. Standard procedures of culture bacteria and identification were applied [10,11].

The Chi-squared test with Yates correction was used for analysis. Significance was established at 5% ($p \leq 0.05$).

Approval for the study protocol was obtained from the Ethics Committee of the Medical University of Białystok.

Results

Tab. 1 presents the results of bacterial composition isolated from the hard palate and dorsum of tongue in 57 denture wearer patients aged 40-83 (mean 66.4 ±11.7) and 38 patient non- wearing dentures, 19-76-year-old (mean 49.9 ±16.0). Isolation frequency (%) in relation to number of patients in the two groups was comparable for all Gram-positive and Gram-negative bacteria with the exception of *Enterobacter cloacae* and *Citrobacter freundii*. These species were isolated only from patients without dentures. No growth of *Streptococcus intermedius* species observed in the mucosal palate swabs of the patients in both groups (*Tab. 1*). Significant difference in the growth rate was seen only for *Haemophilus parainfluenzae*, which isolated most frequently from the tongue (15/57; 26.3%) as compared to palate mucosa (6/57; 10.5%) ($p<0.05$); in patients with dentures.

Table 2. **Isolation frequency (%) of bacteria in denture plaque according to clinical status of patients**

Bacterial species	Isolation frequency (%)			
	Cancer patients (n=16)	Non-cancer patients (n=6)	Diabetic patients (n=19)	Non-diabetic patients (n=16)
Gram-positive bacteria				
Gram-positive cocci				
Staphylococcus spp.	56.3	0	52.6	6.3
S. aureus	37.5	0	0	0
Coagulase-negative	18.8	0	52.6	6.3
Streptococcus spp.	81.3	100.0	84.2	100.0
S. mitis	25.0	16.7	21.0	18.8
S. sanguis	18.8	33.4	10.5	18.8
S. oralis	6.3	0	5.3	0
S. salivarius	12.5	33.4	15.8	37.5
S. vestibularis	18.8	16.7	31.6	25.0
Gram-negative bacteria				
Gram-negative cocci				
Neisseria spp.	25.0	33.4	57.9	62.5
N. mucosa	12.5	0	0	12.5
N. sicca	0	16.7	10.5	12.5
N. subflava	12.5	16.7	31.8	37.5
N. flavescens	0	0	15.8	0
Gram-negative rods				
Haemophilus parainfluenzae	6.3	33.4	5.3	18.8
Enterobacteriaceae spp.	43.8	0	15.8	25.0
Escherichia coli	6.3	0	15.8	6.3
Klebsiella pneumoniae	31.3	0	0	12.5
Citrobacter freundii	6.3	0	0	0
Enterobacter cloacae	0	0	0	6.3
Pseudomonas aeruginosa	18.8	16.7	0	6.3

The aerobic bacteria were isolated with the same frequencies ($p>0.05$) from the oral cavity of patients with and without dentures. Gram-positive cocci (*Staphylococcus* spp. and *Streptococcus* spp.) more often isolated than Gram-negative cocci (*Neisseria* spp.) from both the palate and tongue of patients with and without dentures ($p<0.05$). Among *Streptococcus* spp., *S. mitis* species predominate in the tongue and *S. salivarius* and *S. vestibularis* in the palate of both the groups of patients ($p<0.05$). Bacteria isolated from denture plaques were frequently equal to that isolated from the palate mucosa of 57 patients with dental prosthetic (see *Tab. 1*), with the exception of *Neisseria* spp. which isolated more often from the palate (41/57; 71.9%) than from denture plaque (27/57; 47.4%) ($p=0.05$).

Thorough analysis of isolation frequency of different bacterial species from denture plaques in diabetic and non-diabetic patients, and in patients with and without gastrointestinal cancer shown in *Tab. 2*. Significant difference seen in the composition of bacteria isolated from the denture plaques in patients with and without gastrointestinal cancer. Either no staphylococci nor enteric bacilli isolated from patients without cancer. These bacteria were most frequently isolated from patients with cancer (*Tab. 2*). *Staphylococcus* spp. were more frequently isolated from diabetic patients (10/19; 52.6%) compared to non-diabetic patients (1/16; 6.3%) ($p=0.0032$).

The denture associated stomatitis was observed in 37/57 (64.9%) patients with dental prosthetics. Whole analysis of bacterial composition concerned the number and percent of isolated bacteria from palatal mucosa and denture plaques show no significant difference in patient with and without denture associated stomatitis with the exception of *Neisseria* spp. (*Tab. 3*). *Neisseria* spp. strains more frequent were isolated from palate (73.0%) than denture plaque (51.4%) ($p<0.05$).

Candida albicans was also isolated in 29/37 (78.4%) patients with denture associated stomatitis (data not shown). Among 8/37 (21.6%) patients with denture stomatitis without *Candida albicans*, *Streptococcus* spp. (7/8; 87.5%), *Neisseria* spp. (5/8; 62.5%) and only one species of each (12.5%) *Staphylococcus aureus*, *S. epidermidis*, *Haemophilus parainfluenzae* and *Pseudomonas aeruginosa* were detected.

Discussion

A significant proportion of the adult population wears complete or partial dentures. The factors associated with tooth loss-dental caries, loss of periodontal support, a history of dentoalveolar trauma, a history of dental care – are additive over time, thus denture wearing is more associated with an older population [12]. The oral conditions particularly associated with the wearing of dentures is denture associated stomatitis (DAS)

Table 3. **Incidence rate of aerobic bacteria in denture plaque and palatal mucosa of patients with denture associated stomatitis**

	Number and (%) of bacterial strains			
	Stomatitis (37)		**No-stomatitis** (20)	
	Denture	Palate	Denture	Palate
Gram-positive bacteria				
Staphylococcus spp.	14 (37.8)	9 (24.3)	6 (30.0)	3 (15.0)
Streptococcus spp.	34 (91.9)	34 (91.9)	17 (85.0)	19 (95.0)
Gram-negative bacteria				
Neisseria spp.	*19 (51.4)	*27 (73.0)	8 (40.0)	14 (70.0)
Haemophilus parainfluenzae	5 (13.5)	4 (10.8)	2 (10.0)	2 (10.0)
Enterobacteriaceae	8 (21.6)	5 (13.5)	6 (30.0)	5 (25.0)
Pseudomonas aeruginosa	5 (13.5)	4 (10.8)	1 (5.0)	1 (5.0)

*$p<0.05$

[1-8] or denture related stomatitis [13]. According to Nikawa et al. [13], the term „denture related stomatitis" would be preferable to „denture induced stomatitis" [9,12], since the inflammation of (palatal) mucosa is not induced by the denture, but by wearing the denture or by plaque on the denture. The term "plaque on denture" should by used instead of the term "denture plaque" which used throughout the literature [2,4,12,13], because the microbial flora and its pathogenicity of denture plaque resembles those of plaque formed on the tooth surface, so called dental plaque [13].

Denture plaque has not been studied to the same extent as dental plaque, and although there are many similarities in microbial composition, there are some significant differences [12]. There have been relatively few studies on denture plaque microbiology, the bulk being carried out in the 1980s. Images of denture plaque from the upper fitting surface reveal the presence of a pellicle, a typical biofilm morphology of columnar microcolonies, surmounted by occasional epithelial cells from the maxilla [12,14]. The composition has been deemed similar to dental plaque particularly that on the occlusal surfaces [12,15].

Recent studies are few [12,16]. In published works, the isolates obtained are often a direct consequence of the isolation media used. Many publications focus on *Candida* [12,13]. Thus other groups of organism may be overlooked. This is particularly true for the obligate anaerobes, which are important if oral malodour is the focus of a study [12,16,17].

Some microorganisms which are unusual in the oral microbiota but have been isolated from dentures include respiratory pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *H. parainfluenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* [16-20]. In some studies, 48% of dentures sampled harboured members of *Enterobacteriaceae* [19,20]. Inhalation pneumonia is a common cause of death amongst the elderly debilitated, thus the role of the denture in harbouring such potential pathogens may be significant.

A variety of potential respiratory pathogens had colonized the dentures of our examined patients, the predominant one being *Staphylococcus* spp. (35.1%), among them *S. aureus* contribute to 30.0%. The other putative respiratory pathogens were as follows: *H. parainfluenzae* and *K. pneumoniae* (12.3% each), *S. aureus* (10.5%), *E. coli* and *P. aeruginosa* (8.8% each) and *Enterobacter cloacae* (1.8%). However, no *H. influenzae*, *S. pneumoniae* or *Proteus* spp. were detected. Potential respiratory pathogens were found to have colonized the denture surfaces in 13/57 (22.8%) of the patients.

Our study demonstrated that *Staphylococcus* spp. was isolated more frequently from denture plaques of diabetic patients (52.6%) compared with non-diabetic patients (6.3%) ($p<0.05$). *Staphylococcus* spp. and Gram-negative enteric bacilli were isolated more often from denture plaque (56.3% and 43.8%, respectively) and also from tongue dorsa (50.0% and 31.2%) and palate mucosa (56.3% and 37.5%) in cancer patients than in non-cancer patients (staphylococci and enteric bacilli were not detected) ($p<0.05$). We are suggesting that denture plaque and tongue dorsa can function as a reservoirs of potential respiratory pathogens.

Sumi et al. [21] concluded that denture plaque can function as a reservoir of potential pathogens to facilitate colonization in the pharynx, and it is suggested that denture hygiene status is a significant factor in promoting pharyngeal bacterial colonization. It has been suggested that the tongue surface may also constitute an additional, and possibly more stable, reservoir of respiratory pathogens [22].

The results of this study showed no significant differences in incidence rate of aerobic bacteria in denture plaques and palatal mucosa of patients with and without denture associated stomatitis (DAS). Among our wearer patients 64.9% were with DAS. *Candida albicans* was the most common species associated with DAS (78.4%; data not shown). Our previous study showed that DAS was associated with 94% of yeast isolated, while in wearer subjects and healthy adults (without denture) yeasts have been detected in 75% and 41%, respectively [6]. In 8/37 (21.6%) of our patients with DAS free *Candida albicans* we isolated bacteria such as *Staphylococcus aureus*, *S. epidermidis*, *Haemophilus parainfluenzae*, *Pseudomonas aeruginosa*, *Streptococcus* spp. and *Neisseria* spp. In addition to these bacteria *Enterobacteriaceae* with *C. albicans* were isolated from denture and palate of DAS patients and patients with removable dentures without DAS.

Other authors also showed that DAS was caused by extra-oral species, such as *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp. [1,2,8,9,16]. However, only a strong correlation has been shown for *Candida albicans* and *Staphylococcus aureus* [8]. Moreover, *Streptococcus* spp. and several genera

of anaerobic bacteria (e.g. *Veillonella*, *Lactobacillus*, *Prevotella* and *Actinomyces* spp.) have also been isolated from lesions of patients with DAS [4,9].

There have been relatively few studies on mixed bacterial-fungal biofilms generated in vitro [4]. Different microbial species have frequently been reported to be associated with DAS, however the interactions between bacteria and yeast in the oral cavity have been recognised for several years [24]. Interestingly, co-aggregation studies have shown that *C. albicans* colonization can be aided by primary colonizers such as streptococci [25].

Dorko et al. [7] showed *Candida*-associated denture stomatitis demonstrated in 71.25% examined patients with partial or total dentures. Diabetes mellitus, malignant diseases, chemotherapy, radiotherapy, and broad-spectrum antibiotic therapy were identified by the authors [7] as some of the large number of factors predisposing patients to stomatitis prothetica. Shulman et al. [23] showed that denture stomatitis prevalence is associated with the amount of tissue covered by dentures, low vitamin A levels, cigarette smoking, and constant denture wear. These conclusions were found from a large USA probability sample from the National Health and Nutrition Examination Survey, 1988-1994 (NHANES III); oral examinations (without microbiology) were performed on 3450 individuals 18-90+ years of age [23].

Denture associated stomatitis is usually caused by poor denture hygiene [12], although may be worsened by immunosuppression (e.g. HIV disease) [4,12]. The presence of a denture on the oral mucosa by itself alters the local environmental conditions due to the inaccessibility of saliva and lack of mechanical cleaning by the tongue. Hence, dentures act as reservoirs that harbour *Candida* spp. within a mixed species of bacterial biofilm.

In conclusion, denture hygiene is the obvious method for ensuring that the denture remains clean. There are several oral hygiene products available for use by denture wearers.

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Effect of sodium fluoride on the morphological picture of the rat liver exposed to NaF in drinking water

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Abstract

Purpose: **Due to its efficacy in caries prophylaxis and easy application, sodium fluoride (NaF) is still used for caries prevention in the form of fluoridated drinking water, fluoride tablets, fluoridated salt or milk. Effect of fluorides on various metabolic levels in hard and soft tissues, namely respiration as well as carbohydrate, protein, enzymatic and vascular metabolism, can disturb detoxication of fluorine compounds administered orally. The study objective was morphological examination of the liver of young and mature rats exposed to NaF in drinking water from conception till maturity, as well as after its withdrawal.**

Material and methods: **In the initial stage of the experiment, 30 female Wistar rats, 180-200 g body weight, were divided into 3 groups: one control and two experimental groups (I, II). Female rats in the experimental groups received fluorine in aqueous solutions of sodium fluoride (NaF) at a concentration of 10.6 mg NaF/dm^3 (group I) and 32.0 mg NaF/dm^3 (group II).**

Results: **The pathomorphological changes observed in the liver, particularly of young rats exposed to fluorides at superoptimal doses can help determine to what degree oral fluoride caries prevention is safe and whether it should be implemented. The transitory nature of pathomorphological changes in hepatocytes indicates adaptive potentials or defence mechanisms against orally administered sodium fluoride.**

Key words: fluoride, liver, rat, morphological picture.

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Introduction

Due to its efficacy in caries prophylaxis and easy application, sodium fluoride (NaF) is still used for caries prevention in the form of fluoridated drinking water, fluoride tablets, fluoridated salt or milk [1]. Effect of fluorides on various metabolic levels in hard and soft tissues, namely respiration as well as carbohydrate, protein, enzymatic and vascular metabolism, can disturb detoxication of fluorine compounds administered orally [2-4].

Fluorine, considered to be one of the environmental toxins [5], does not occur free in nature but thanks to high affinity for the ions of calcium, sodium, magnesium and tin it forms chemical compounds with them, which are more or less soluble in water [6].

Because of good solubility in water, easy absorption from the alimentary tract as well as for economic reasons, sodium fluoride (NaF) is the most commonly used compound in collective endogenic oral caries prophylaxis. During oral exposure, it can positively affect the oral environment. However, when consumed with food via the alimentary tract it can change, depending on dose and exposure time, cell and tissue metabolism in the further stages [1]. The organ that reacts rapidly to xenobiotics reaching the body from the outside is the liver. It is there where detoxication processes take place and the resulting pathomorphological changes are the response to the orally administered preparation.

Objective

The study objective was morphological examination of the liver of young and mature rats exposed to NaF in drinking water from conception till maturity, as well as after its withdrawal.

Material and methods

In the initial stage of the experiment, 30 female Wistar rats, 180-200 g body weight, were divided into 3 groups: one control and two experimental groups (I, II). Female rats in the

Figure 1. **Picture of a 30-day-old rat hepatocyte, control group. H+E stained. Magn. x400**

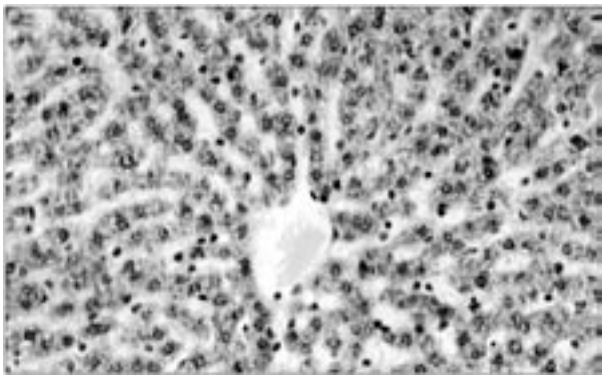
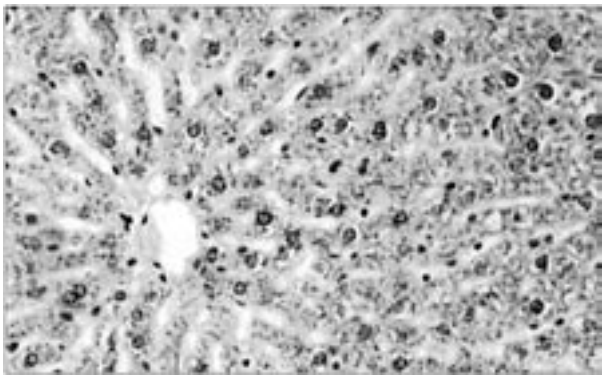


Figure 2. **Picture of a 30-day-old rat hepatocyte, examined group II (10.6 mg NaF/dm^3). Vacuolar degeneration. H+E stained. Magn. x400**



experimental groups received fluorine in aqueous solutions of sodium fluoride (NaF) at a concentration of 10.6 mg NaF/dm^3 (group I) and 32.0 mg NaF/dm^3 (group II), corresponding to a dose of 1.2 mg F/kg b.w. (group I) and 3.6 mg F/kg b.w. (group II). Sodium fluoride (NaF), (crystalline powder Natrium fluoride, Sigma, Germany) was dissolved in tap water. After a two-week adaptation period, during which the rats drank an average amount of 50 ml of water, the females were covered. They received NaF with drinking water 3 days before covering, during pregnancy and lactation. On day 30 of life, young rats were separated from mothers but were still given sodium fluoride with drinking water in the concentrations as above, ad libitum. Control animals received tap water ad libitum, in which the level of fluorine did not exceed 0.2 mgF/dm^3. On day 90 of life, NaF was withdrawn in groups I and II, and tap water alone was administered to all the animals. The experiment was terminated on day 120 of life.

Of various experimental models of intoxication with fluorine compounds, the one with fluoride administration via the alimentary tract in rat is the closest to oral fluoride prophylaxis in man. The concentration of 10.6 mg NaF/dm^3 drinking water, applied in the current study, results from a 10-fold lower sensitivity of rat to fluorine [7] and is considered to be the 'optimum' fluorine level for rat as compared to the acceptable level for man being 0.8-1 mg F/dm^3 of drinking water. The choice of NaF can be justified by its easy solubility in water, easy absorption in the alimentary tract and simple application in oral, both collective and individual, caries prophylaxis. Deionised water was not used to dilute NaF, as we believe that all micro- and macroelements contained in tap water are necessary for metabolic processes in tissues.

The animals were kept in standard environmental conditions [8]. The young were subjected to the action of fluoride from conception, through the foetal period, nest period (till day 30 of life) and maturity (day 90). After 90 days of exposure, NaF was withdrawn. The experiment was terminated on day 120 of life. Rats were fed on standard granulated LSM diet.

90 young rats were included in the experiment, 5 in each study subgroup. The animals were weighed and subjected to autopsy at the following age intervals: day 4 (newborn), day 14 (young rat fed on mother's milk); day 30 (young rat, receiving mother's milk, drinking water and standard diet from day 14 to day 30 of life – on that day, the young were separated from mothers but the experimental model was continued), day 60 (mature rat), day 90 (adult rat) and day 120 (NaF was not applied for 30 days). In all experimental animals, sections were collected from the anterior lobe of the liver. The material for light microscopic examination was fixed in 10% formalin, and embedded in paraffin cubes, cut on a rotary microtome into 7 μ sections, and stained with haematoxylin and eosin (H+E) [9].

The weight of the selected animals was monitored. The experiment was approved by the Bioethics Committee, Medical University of Białystok.

Results

Histological findings H+E

Control group (animals drinking tap water)

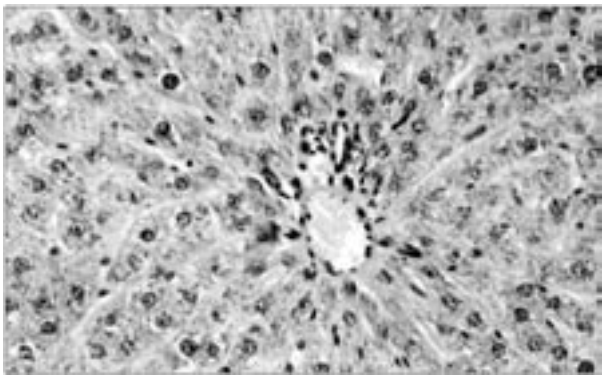
In the animals dissected on day 4 of the experiment, hepatocytes in all fields of vision formed chaotic spatial arrangements. Pictures of the liver showed the predominance of blood vessels and very numerous haemopoietic system cells that formed islets. Hepatocytes had a foamy, acidophilic cytoplasm. Cell nuclei were well stained, with distinct chromatin lumps and nucleoli. In 14-day-old rats, hepatocytes showed a distinct trabecular arrangement and contained acidophilic cytoplasm with markedly stained nuclei and nucleoli. In most fields of vision, lobules were distinct. The haemapoietic cells sporadically formed islets. In 30-day-old animals, the liver structure was already mature (*Fig. 1*). In 60-, 90- and 120-day rats, the structure of the organ was typical for adult animals. Slight vacuolar degeneration could be sporadically seen in the peripheral parts of the lobules.

Group I

(NaF concentration in drinking water – 10.6 mg/dm^3)

In the animals dissected on day 4, pictures of the liver did not differ markedly from those seen in the control group. In some fields of vision, hepatocytes contained vacuoles. In the livers of 14-day-old rats, in some hepatocytes the cell membrane was blurred. Blood vessels were slightly dilated. In 30-day-old

***Figure 3*. Picture of a 120-day-old rat hepatocyte, examined group II (10.6 mg NaF/dm^3), a few cells with features of vacuolar degeneration. H+E stained. Magn. x400**



rats, vacuolar degeneration-type changes occurred. Necrotic lesions were sometimes seen in hepatocytes (microfocal lesion). In 60- and 90-day-old rats the liver had a normal structure. In the peripheral parts of rare lobules slight vacuolar degenerations were found. In 120-day-old rats in this group, i.e. 30 days after NaF withdrawal, the vacuolar degenerations persisted, particularly in hepatocytes of the peripheral lobules.

Group II

(NaF concentration in drinking water – 32.0 mg/dm^3)

In this group, morphological changes in the liver were more pronounced in all the animals regardless of the time of dissection. Already in the livers of 4-day-old rats a large number of hepatocytes with vacuoles in the cytoplasm were seen. Blood vessels were dilated and filled with clotted acidophilic fluid. On day 14, hepatocytes showed features of damage, visible in the structure of cell and nuclear membranes. In the vicinity of blood vessels, inflammatory infiltration of neutrophilic granulocytes was observed. In the livers of 30-day-old rats, the changes were further intensified, and hepatocytes showed distinct vacuolar degeneration and micronecrotic foci (*Fig. 2*). In 60-day-old rats, the picture of intoxication in the liver was still present. In the livers of 90-day-old rats, the changes were becoming weaker. However, vacuolar degeneration-type changes still persisted in the livers of 120-day-old rats despite NaF withdrawal (*Fig. 3*).

Discussion

According to research surveys conducted by the International Research Agency for Fluorination, the toxicity of fluorine compounds is frequently ignored by medical doctors, dentists and paramedical staff involved in healthcare. Therefore, measures should be taken to obtain undoubted benefits resulting from the application of fluorine compounds in caries prevention and to minimize any side-effects [6,10-14].

Assuming that the liver is involved in the metabolism of toxic compounds produced during systemic transformations and exogenous toxins getting to the organism from the environment, we could expect to find both pathomorphological and metabolic changes as reactions to NaF. Therapeutics or toxins, to which NaF, depending on its dose, can be included, are likely to impair liver function and induce morphological changes in the liver [15,16-23]. Hepatotoxic action is manifested by cell respiration disorders that interfere with oxidation and reduction mechanisms, by impairment in protein, carbohydrate and lipid metabolism and by disturbances in intra- and extracellular transport. In consequence, whole cell or its cytoplasmic organelles can be damaged. Most frequently the damage is expressed as parenchymal vacuolar degeneration, necrosis of hepatocytes or disorders in the activity of metabolic enzymes [15,24-29]. In the livers of newborn rats in group II, slight changes were found in Browicz-Kupfer cells at higher fluoride concentration. Distinct blood vessel dilation could suggest metabolic disturbances in the liver, thus indicating a potential toxic effect of fluoride on the organ as early as in the foetal period. The livers of 14-day-old rats exposed to NaF in group I and II, receiving fluorine only with mother's milk, showed vacuolar degeneration-type changes, damage or blurring of cell or nuclear membrane and vessel dilation. The changes were more pronounced in group II, i.e. at higher fluoride concentration.

Lack of distinct morphological and enzymatic changes in the livers of 14-day-old animals of group I (lower NaF concentration) can be explained by the protective role of mother's milk [30]. Since morphological and ultrastructural changes were most pronounced in the livers of 30-day-old rats, we would like to discuss them more widely and compare with the findings of other authors. Up to day 30 of life, the rats stayed in nests, receiving both mother's milk, standard diet and NaF-enriched water to drink, which resulted in combination of drinking water fluorides with mother's milk fluorides. Already in group I, at the lower NaF concentration, apart from vacuolar degeneration also micronecrotic foci was observed. In group II, at the higher NaF concentration, vacuolar degeneration and numerous micronecrotic foci were seen to multiply as compared to group I.

After NaF withdrawal the changes in the liver in both groups were subsiding. The amount of glycogen increased in hepatocytes, and cell nuclei and endoplasmic reticulum were normal in appearance. Only mitochondrial polymorphism was maintained and damaged endothelial blood cells were sporadically seen.

Endoplasmic reticulum reacts rapidly to the action of toxic compounds [31]. Its rough component undergoes vacuolization (vacuolar degeneration) and loses ribosomes, which leads to a decrease in RNA. These changes are associated with the impairment in protein synthesis within the cell [32]. The smooth part of the endoplasmic reticulum can also be subject to vacuolisation or proliferation, which causes a considerable decrease in the count of glycogen granules in the affected sites [27,33]. This has been confirmed by our previous ultrustructural findings [34]. The electron microscope examinations revealed dilation of channels of the rough endoplasmic reticulum in 30-day-old group II animals, i.e. those exposed to the higher concentration of fluoride ions. Similar changes in the liver found in the rough endoplasmic reticulum have been described by Lavrushenko [34]. Detachment of ribosomes from the reticular membranes may indicate disorders in protein production within the cell, which may be caused, as Pasternak suggests, by a drop in tRNA

aminoacylation in the presence of fluoride ions [35]. A decrease in proteins produced by the rat liver at the time of exposure to fluoride has been also described by Wędzisz [36].

Additionally, in group II rats older than 30 days, the sinusal lumen of the liver sometimes showed collagen bundles accompanied by micronecrosis observed in the morphological examination, which could suggest the beginning of liver fibrosis.

From day 30 till day 90 of NaF administration, the pathomorphological changes showed a gradual decrease in intensity and only considerable dilation of blood vessels with endothelial swelling was observed. Taking into account a continuous exposure to NaF, the decrease could be the result of adaptive mechanisms of the organism to fluoride, which have been discussed by Machoy-Mokrzyńska [37]. Hepatic hyperaemia after administration of acetate acid to rats has been observed by Luty [38]. In some systemic diseases, hyperaemia seems to be beneficial, e.g. in myocarditis. The findings of laboratory and epidemiologic studies conducted during water fluoridation period suggest that the mortality rate due to heart infarct may have decreased due to fluorine compounds present in drinking water [39,40].

Some literature reports as well as our own findings (unpublished) seem to prove that fluorine accumulates in the liver [41]. Its blood level depends on fluoride supply, which refers to all fluorine forms in blood serum [30]. As revealed by Chlebna-Sokół, due to high homeostasis of the serum, blood fluoride levels remain constant even in the case of overdosage [42].

The NaF-induced morphological changes in rat hepatocytes create a picture similar to those observed after intoxications with other toxic compounds administered to experimental animals. It can be assumed that the liver is involved in detoxication of excessive fluorine doses. After NaF withdrawal, the changes in the liver in both groups were subsiding. The glycogen count increased, but in a considerable number of cells vacuolar degeneration persisted.

Remission of most pathomorphological changes after NaF withdrawal may suggest their transitory nature. However, at the time of exposure sodium fluoride affects the development of the organism both in the prenatal and postnatal period of experimental animals.

Conclusions

Although the findings obtained for rats cannot be directly referred to the human body, the pathomorphological changes observed in the liver, particularly of young rats exposed to fluorides at superoptimal doses can help determine to what degree oral fluoride caries prevention is safe and whether it should be implemented. The transitory nature of pathomorphological changes in hepatocytes indicates adaptive potentials or defence mechanisms against orally administered sodium fluoride.

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Effect of chlorhexidine mouthrinse on cathepsin C activity in human saliva

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Abstract

Chlorhexidine is an active agent commonly used against dental plaque in the mouth apart from fluorides applied to prevent caries. It is contained in toothpastes and mouthrinses.

Purpose: The aim of the study was to assess the effect of mouthrinses containing chlorhexidine digluconate on the activity of cathepsin C in human saliva.

Material and methods: Material for analyses contained mixed saliva samples collected at rest, directly into test tubes (Z PS type, Medlab) at least 2 hours after meal from 40 subjects (dentistry students; 30 women and 10 men), aged 19-24. Saliva was collected before the preparations were applied after rinsing the mouth with distilled water and following a single use of the preparations according to the producer's instructions, 8 samples for each preparation.

Results: The decrease of cathepsin C was observed for each preparation, but was the greatest after mouth rinsing with Kin Gingival (65.08%) and Corsodyl (58.00%).

Conclusions: The current study confirms this assumption by finding a decrease in cathepsin C activity after the use of chlorhexidine mouth rinses.

Key words: cathepsin C, chlorhexidine, human saliva, mouthrinses.

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Introduction

Chlorhexidine is an active agent commonly used against dental plaque in the mouth apart from fluorides applied to prevent caries [1-4].

Chlorhexidine is the longest and the most frequently used antibacterial and anti-inflammatory agent, being the focus of research as far back as the 50s of the previous century. It is considered to be one of the most effective antiseptics, decreasing dental plaque formation and inhabiting the development of gingivitis even when mechanical cleaning has been neglected. It is contained in toothpastes (Elgydium, Lacalut, KIN-Gingival) and mouth rinses (Corsodyl, Oral-B, Peridex, Parogencyl, Paroplak, Eludril, Oralsept, KIN-Gingival, Protefix, Gluxonit) [5].

Chlorhexidine, the cationic form of bis-biguanidine, occurs as gluconate or acetate. Charged positively, it shows high affinity for negative ions found in cell membranes of microorganisms. Chlorhexidine is more effective against the cell membranes of Gram-positive bacteria as they have a much higher charge than the Gram-negative ones. The hydrophobic part of chlorhexidine reacts with the structures of the bacterial cell membrane, disturbing its integrity and function. At a low concentration, molecules of the antiseptic bind to phosphate groups of lipopolysaccharides and to carboxy groups of proteins of the cell wall. This action interferes with cellular transport (potassium ions, amino acids and nucleotides) and disturbs metabolic processes [3]. Chlorhexidine indirectly affects the enzymatic function of dehydrogenase and ATP-ase present in the cell wall of bacteria [6].

At high concentrations of chlorhexidine, the cell membrane gets disrupted, which results in osmotic imbalance, escape of cytoplasmic components and cell death [7]. It can be thus assumed that high levels of this antiseptic exert a bactericidal effect, while low concentrations show a bacteriostatic action. Moreover, chlorhexidine binds to negatively charged mucous cells of the oral cavity, but as they differ in structure from the bacterial cells they remain intact. Bound to the mucous membrane or saliva proteins, chlorhexidine is gradually released in

Table 1. **Mean values of the study parameters in the overall research material**

	KIN-Gingival 0.12% chlorhexidine digluconate		**Corsodyl 0.1% chlorhexidine digluconate**		**Protefix 0.1% chlorhexidine digluconate**		**Eludril 0.1% chlorhexidine digluconate 0.5% chlorobutanole**		**Control distilled water**	
	Mean ±SD		Mean ±SD		Mean ±SD		Mean ±SD		Mean ±SD	
	Before	After	Before	After	Before	After	Before	After	Before	After
Cathepsin [nmol/ml]	0.1724 ±0.07	0.0602 ±0.02	0.0844 ±0.21	0.0346 ±0.03	0.0854 ±0.02	0.0542 ±0.01	0.0616 ±0.09	0.0496 ±0.06	0.1415 ±0.12	0.18875 ±0.08
Protein [mg/ml]	0.1506 ±0.06	0.125 ±0.03	0.2376 ±0.07	0.29 ±0.07	0.2068 ±0.08	0.1626 ±0.08	0.1378 ±0.05	0.1634 ±0.08	0.98725 ±0.19	0.9725 ±0.11

the presence of Ca+2. Thus, for a certain period of time, the oral cavity becomes a chlorhexidine reservoir, which prolongs its chemical activity in preparations [3,6,7].

In the inflammatory processes in the mouth, lysosomal proteolytic enzymes are released, inducing periodontal disorders. These enzymes include cathepsin C (dipeptidyl-peptidase I), an exopeptidase, which splits off dipeptides from the N-terminal of peptides [8]. Cathepsin C belongs to exogenic salivary peptidases, cleaves off p-nitroaniline (pNA) from dipeptide p-nitroanilides, has a cystein catalytic site and to be active needs chloride anions. It is also believed to have transferase properties [9]. Since no literature data are available on the effect of chlorhexidine gluconate on the activity of cathepsin C, we have decided to examine this relationship.

Objective

The aim of the study was to assess the effect of mouthrinses containing chlorhexidine digluconate on the activity of cathepsin C in human saliva.

Material and methods

Material for analyses contained mixed saliva samples collected at rest, directly into test tubes (Z PS type, Medlab) at least 2 hours after meal from 40 subjects (dentistry students; 30 women and 10 men), aged 19-24. They were divided into 5 groups, each group receiving different preparation, and control group. The students were healthy, non-smoking, with no active foci of caries. In 4 groups of 8 students, chlorhexidine mouthrinses were used. In group 5 (control), distilled water was applied as a mouth rinse. Saliva was collected before the preparations were applied after rinsing the mouth with distilled water and following a single use of the preparations according to the producer's instructions, 8 samples for each preparation. Test-tubes with the saliva samples were frozen immediately and stored at a temp. of -18°C ÷ -24°C. After defrosting, the activity of cathepsin C (EC 3.4.14.1) was determined in non-fractionated saliva according to Plant with the use of Gly-Phe-pNA substrate. [10]. The enzyme activity was measured by assessing the amount of the obtained product and expressed in nmol/ml [11]; Bradford method was used to assess protein content [12].

The following preparations containing chlorhexidine digluconate were used: KIN-Gingival (0.12%), Corsodyl (1.1%), Protefix (0.1%), Eludril (1.1%). The study was approved by the local Bioethics Committee.

The results were subjected to statistical analysis using Statistica programme 6.0, StatSoft. Normality of distribution of the respective variables was determined with Kolmogorow-Smirnow test. To compare mean values of the respective parameters before and after application of the preparation, Student's t-test was employed for dependent variables in the case of normal distribution parameters and a non-parametric sign test for dependent variables in the case of abnormal distribution parameters.

Results

The average values of the study parameters, cathepsin C activity and the amount of salivary protein calculated for all the study subjects have been presented in *Tab. 1*. Gender was not taken into account as the parameters did not differ significantly between women and men. Since all the preparations contained chlorhexidine digluconate in similar concentrations, they have been listed according to cathepsin C decrease caused by their action. The decrease was observed for each preparation, but was the greatest after mouth rinsing with KIN-Gingival (65.08%) and Corsodyl (58.00%). Protefix showed similar effects (36.53%). The slightest reduction in cathepsin C activity was found after the application of Eludril (19.48%), which may be explained by the fact that 0.5% chlorbutanol was added to this rinse. Most of the chlorhexidine preparations used caused a slight decrease in the amount of salivary protein. However, the results were not statistically significant.

Discussion

Chlorhexidine is one of the most effective antiseptics. It inhibits dental plaque formation and gingivitis even when mechanical cleaning has been neglected. It is contained in toothpastes and daily use mouthrinses. Chlorhexidine digluconate is the active component of the rinses. Our current findings demonstrate a reduction in cathepsin C activity after application of chlorhexidine-containing preparations. Previously, we demonstrated a distinct and statistically significant reduction in the enzyme activity due to fluoride preparations [13].

The decrease was larger for amino fluorides (72.6% for Elmex green liquid), probably due to higher bioactivity of amino

fluorides on cathepsin C, compared to chlorhexidine. The effect of fluoride preparations on salivary enzymes has been demonstrated in many studies. Kaczmarek [14-16], who studied the activity of such salivary enzymes as alpha-amylase, peroxidase and myeloperoxidase, obtained their reduction in the environment of fluoride ions suggesting high biochemical activity of the latter. A more substantial decrease in cathepsin C activity after amino fluorides demonstrated in our earlier study than after chlorhexidine seems to confirm a specific mechanism of fluorides involving inhibition of intra- and extra-cellular enzymes.

Literature data on cathepsin C and its effect on oral health are lacking and hence no discussion with the findings of other authors is possible. Research reports have revealed that the activity of cathepsins B, L, D intensifies in periodontal inflammatory states, suggesting involvement of these enzymes in tissue degradation, damage to collagen, elastin and fibronectin [17--20]. Only Etemadzadeh et al., who investigated chlorhexidine and aminofluoride-based preparations, found no changes in the activity of lysosomal enzymes, saliva pH or saliva secretion rate. However, they noted a smaller amount of dental plaque due to chlorhexidine application compared to aminofluorides [21]. The effect of chlorhexidine on bacterial plaque has been already investigated in clinical settings.

Dental plaque has the potential to bind substantial amounts of the antiseptic, whose proper concentrations may cause plaque disintegration and splitting from the enamel surface. Application of chlorhexidine varnish reduces colonisation of *Streptococcus* mutans in dental plaque [4]. Particularly sensitive even to low doses of chorhexidine are the bacteria *Streptoccocus* mutans, *Fusobacterium nucleatum*, *Streptoccocus sanguinis*, *Actinomyces*, *Lactobacillus*, *Enterococcus faecalis*, *Porphyromonas gingivialis*, *Prevotella intermedia*, as well as yeasts (*Candida albicans*), certain dermatophytes and *in vitro* viruses (Herpes simplex, HIV). Chlorhexidine gluconate in a mouthrinse caused a drop in aerobic and anaerobic bacterial cultures, with lower sensitivity of Gram(-) anaerobes [25].

A two-minute mouth rinsing with Skinsept was found to result in a considerable decrease in the number of the respective bacteria or even their elimination. Reduction in the bacterial population on the tongue surface and in saliva ranges between 81% and 99% [26,27].

During local irrigation with 0.3% antiseptic a decrease was observed in periodontal inflammatory symptoms, in dental plaque index, in gingival fluid index, sulcus bleeding index, probing depth and bacterial counts. Besides, chlorhexidine irrigations improve the epithelial attachment level due to the inhibitory effect of chlorhexidine on the enzymes involved in periodontitis: metalloproteinases, cathepsins, elastases [28,29].

Research on the relationship between periodontal diseases and the presence of proteolytic enzymes in the mouth may elucidate a complex mechanism of these diseases.

Conclusions

Considering our findings and the results reported by other authors it can be assumed that mouth rinses for everyday use can improve inflammatory states of periodontal tissues through inhibition of proteolytic enzymes. The current study confirms this assumption by finding a decrease in cathepsin C activity after the use of chlorhexidine mouth rinses.

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Assessment of dental status and oral hygiene in the study population of cystic fibrosis patients in the Podlasie province

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Abstract

Purpose: Cystic fibrosis (CF) is one of the most common genetic diseases worldwide. It is caused by mutations of the gene situated on the long-arm of the 7th chromosome coding *Cystic* Fibrosis Transmembrane Conductance Regulator (CFTCR) which is responsible for the synthesis of cAMP--dependent membrane chloride channel located on the top surface of epithelial cells of exocrine glands. Accumulation of the secretion in the outlet ducts caused by a dysfunction or lack of CFTR proteins leads to abnormal activity of exocrine glands, especially in the respiratory and alimentary tracts. Carbohydrates, the main dietary component, supply energy to the body, but at the same time are the major cariogenic agent. The aim of the current study was to assess dental caries disease and oral hygiene in CF patients in the region of Podlasie.

Material and methods: The study involved 23 patients with cystic fibrosis, aged 2.5-24 years, from the Podlasie Province treated in the Outpatient Cystic Fibrosis Department of the Children's University Hospital in Białystok. Three age groups were distinguished: 1-5, 6-12, 13-24 years. The following were evaluated: caries incidence (percentage of patients with caries CI), caries intensity – based on the mean dmf/DMF score, oral hygiene – based on the dental plaque index (OHI-pl).

Results: The incidence rate of caries was found to be very high both in the CF population and in the control group. In children with mixed dentition it was 100%. For permanent teeth, mean DMF score was 3.55 in group II and 10.9 in group III. In CF patients, dental plaque index was the highest in group III.

Conclusions: In CF patients, there is a serious risk of caries due to severe course of the disease, long-term administration of medications and high carbohydrate diet. CF patients should remain under constant dental care according to the individually designed programmes of oral health promotion and caries prophylaxis.

Key words: dental status, oral hygiene, cystic fibrosis.

Introduction

Cystic fibrosis (CF) is one of the most common genetic diseases worldwide. It is caused by mutations of the gene situated on the long arm of the 7th chromosome coding Cystic Fibrosis Transmembrane Conductance Regulator (CFTCR) which is responsible for the synthesis of cAMP-dependent membrane chloride channel located on the top surface of epithelial cells of exocrine glands [1]. A dysfunction or lack of CFTR proteins leads to disorders in Cl^- transport through cell membranes and increased Na+ and water absorption, resulting in the formation of dense and sticky secretion. Accumulation of the secretion in the outlet ducts leads to abnormal activity of exocrine glands, especially in the respiratory and alimentary tracts [2].

It is estimated that in Poland (like in most European countries) cystic fibrosis occurs in 1/2500 newborns and every 25th person is a carrier of abnormal CFTR gene, responsible for the disease. In the classic form (fully symptomatic), cystic fibrosis patients present with predisposition to bronchitis and pneumonia, failure of the exocrine part of the pancreas, male infertility and elevated sweat chlorine concentration [3,4]. Respiratory pathologies frequently determine the quality and length of life. Carbohydrates, the main dietary component, supply energy to the body, but at the same time are the major cariogenic agent.

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***Table 1*. Incidence of caries in the groups (CI)**

Study subjects		Number of subjects with caries			
		CF patients		Control group	
		n	%	n	%
Number of subjects aged 2.5-5 years with deciduous dentition	4	2	50	2	50
Number of subjects aged 6-12 years with mixed dentition	9	9	100	6	66
Number of subjects aged 13-24 years with permanent dentition	10	9	90	9	90
Overall	23	20	87	17	74

***Table 2*. Mean dmf in deciduous dentition in the study groups**

Group I	Number of subjects (n)		Mean dmf and its respective elements			
			d	m	f	dmf
Deciduous dentition	4	CF patients	3.25	0	0	3.25
	4	Control group	2.75	0	0	2.75

Legend: dmf – mean dmf score in deciduous teeth; d – mean number of deciduous teeth with active caries, m – mean number of deciduous teeth extracted due to caries, f – mean number of filled deciduous teeth

***Table 3*. Mean dmf/DMF score in mixed dentition in the study groups**

Group II	Number of subjects		Mean dmf + DMF and its respective elements										
			D	d	D+d	M	m	M+m	F	f	F+f	DMF	dmf	DMF+dmf
Mixed dentition	9	CF patients	2.33	1.77	4.11	0	0.55	0.55	1.22	0.11	1.33	3.55	2.44	5.99
	9	Controlgroup	0.22	1.22	1.44	0	0.44	0.44	0.55	2.11	2.66	0.77	1.77	2.54

Legend: dmf+DMF – mean dmf+DMF score in mixed dentition, d; D – mean number of teeth with active caries; d – deciduous teeth; D – permanent teeth, m; M – mean number of teeth extracted due to caries, m – deciduous teeth; M – permanent teeth; f,F – mean number of filled teeth; f – deciduous teeth, F – permanent teeth

This may suggest that CF patients are particularly susceptible to dental caries.

The aim of the current study was to assess dental caries disease and oral hygiene in CF patients in the region of Podlasie.

Material and methods

The study involved 23 patients with cystic fibrosis, aged 2.5-24 years, from the Podlasie Province treated in the Outpatient Cystic Fibrosis Department of the Children's University Hospital in Białystok. Three age groups were distinguished: 1-5, 6-12, 13-24 years, based on dentistry literature data and taking dentition type into consideration (deciduous dentition group I, mixed dentition group II, permanent dentition group III). The control group consisted of 23 healthy subjects randomly chosen out of those admitted to the Specialist Dental Clinic, Medical University of Białystok. Each CF patient was matched by age and gender to a control subject. The patients underwent a routine dental examination in artificial light, using a probe and a mirror. The methods used were consistent with the World Health Organization guidelines. Oral hygiene was checked after prestaining of the plaque with Red Cote tablets, Butler. The patients' parents gave their consent for dental examination.

The following were evaluated:

– caries incidence (percentage of patients with caries CI)
– caries intensity – based on the mean dmf/DMF score
– oral hygiene – based on the dental plaque index (OHI-pl according to Green and Vermillion)

Results

The incidence rate of caries was found to be very high both in the CF population and in the control group (*Tab. 1*). In the deciduous dentition group, the incidence rate reached 50% in CF and control children. In children with mixed dentition it was 100% (66% in control) and in children and adolescents with permanent teeth it was 90% (like in control). Only 3 CF patients and 6 healthy subjects were not affected by caries.

The intensity of the caries disease process was expressed as the mean dmf/DMF score (*Tab. 2*, *3*, *4*), which determines caries intensity for all subjects, not only those with caries. For CF patients, mean dmf was 3.25 in group I (deciduous dentition) and 2.44 in group II (mixed dentition). For permanent teeth, mean DMF score was 3.55 in group II and 10.9 in group III, thus being lower than in healthy subjects.

In CF patients (*Tab. 5*), dental plaque index was the highest in group III (1.28). It was lower in mixed dentition patients (0.5), while in patients with deciduous dentition the plaque was not present. Slightly higher values of OHI-pl were noted for the respective control groups.

Table 4. **Mean DMF in permanent dentition in the study groups**

Group III	Number of subjects (n)		Mean DMF and its respective elements			
			D	M	F	DMF
Permanent dentition	10	CF patients	5.8	0.1	5	10.9
	10	Control group	5.5	0.3	7	12.8

Legend: D – mean number of permanent teeth with active caries; M – mean number of teeth extracted due to caries; F – mean number of filled permanent teeth; DMF – mean DMF score in permanent dentition

Table 5. **Oral hygiene index (OHI- pl) according to Green and Vermillion in the study groups**

CF patients	Number of subjects	OHI-pl	OHI-pl			
			<1		≥1	
			n	%	n	
I	4	0	0	0	0	0
II	9	0.55	7	77.77	2	22.23
III	10	1.28	4	40	6	60
Control group						
I	4	0.99	1	25	3	75
II	9	0.81	6	66.66	3	33.34
III	10	1.31	4	40	6	60

Legend: n – number of the study subjects; OHI pl – oral hygiene index (plaque) according to Green and Vermillion

Discussion

Due to its common incidence, dental caries belongs to social diseases. Its main etiological factor is the bacteria most frequently transmitted by parents or caretakers in the early childhood [5]. Carbohydrates provided to the oral cavity together with food are the medium for cariogenic bacteria. As CF patients receive high carbohydrate diet, it is assumed that the cariogenic process can be more intensified in this group of patients. Various species of bacteria break down saccharides to acids, which leads to enamel demineralization and cavity formation. When oral hygiene is insufficient, dental deposits undergo mineralization and being transformed into dental calculus cause parodontosis [6]. CF patients, burdened with numerous ailments from many organs, receive various medications which affect the quality and quantity of saliva secretion [7]. Moreover, their potential cariogenic effect on teeth is increased by sweeteners added to drugs to improve taste [8]. Inhalers contain steroids which after long-term administration may cause oral mycosis, just like antibiotics taken in great amounts. Due to frequent infections of the upper respiratory tract, CF patients often breathe through the mouth, which promotes malocclusions and predisposes to periodontitis and inflammation of oral mucosa. The incidence of caries in the study population of CF patients in the Podlasie Province was high and in most cases comparable to the data reported by Olejniczak et al. for the study population in Poland [9,10]. Consistent with our results was higher caries incidence rate found within the age ranges of 6-12 and 13-24 years in CF patients, as compared to the controls. However, in our study, in the age range of 2.5-5 years, the incidence rate in CF patients was similar to that noted in healthy subjects. A comparison of caries incidence between patients with cystic fibrosis and cow milk intolerance, which is also manifested in malabsorption of nutrients, is more favorable for CF patients. Caries incidence was higher in children with food intolerance both for deciduous and permanent teeth (fp=88.50% i FP=95.20%, respectively) [11]. This may be caused by medications taken and a different type of diet, particularly in the period of maturation. Similarly to other authors, we found lower caries intensity expressed in mean DMF score only in CF patients aged 13-24 years, as compared to the control group [12-14]. Also in the study conducted by Olejniczak et al. on the Polish population, the p/P component for each study group in Podlasie was higher in CF patients than in the respective control group, being the highest in group III=5.8, with a lower mean DMF for these patients compared to healthy subjects [9]. Vitamin and micro- and macroelement therapy instituted in CF patients strengthens enamel and thus makes it more resistant to the caries process. Digestive enzymes taken by CF patients have a beneficial effect on saliva buffer capabilities [15], creating better conditions for the oral ecosystem. CF patients had higher concentrations of calcium, phosphate and potassium ions [16], increased pH of the saliva and greater buffer capacity [17], which suggests a simultaneous effect of these parameters on the reduction in caries intensity in CF patients.

Long-term antibiotic therapy in CF patients may inhibit the quantity of cariogenic bacteria in the oral cavity [15,18], the assumption confirmed by the mean DMF value for this group and a smaller predisposition to periodontitis. In the clinical study on the Podlasie population, like in other studies conducted in Poland and Belgium, CF patients demonstrated better clinical condition of periodontal tissues than healthy subjects, i.e. smaller bleeding from the gums and less or no dental calculus [1,8,10]. This is confirmed by lower values of oral hygiene index OHI-pl noted in the present study, despite poor awareness of the patients and their caretakers concerning oral ailments, observed in the accompanying surveys.

Conclusions

1. In CF patients, there is a serious risk of caries due to severe course of the disease, long-term administration of medications and high carbohydrate diet.

2. CF patients should remain under constant dental care according to the individually designed programmes of oral health promotion and caries prophylaxis.

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Assessment of dentition status and oral hygiene in first year dental students, Medical University of Białystok

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Abstract

Purpose: **Caries, a social ailment, is one of the diseases of civilization of the 20th century. In Poland, the incidence rate of caries is very high both in the young and adults. The major etiological factors of caries are: improper oral hygiene, diet based on carbohydrate-rich and highly processed food products, neglect of prophylaxis and dental check-up. The aim of the study was to assess dental status and oral hygiene of the first year dental students, Medical University of Białystok, through the analysis of the chosen caries and dental plaque indices.**

Material and methods: **The study group consisted of 70 first year dentistry students, including 50 women and 20 men, aged 19-23 years. Dentition status and oral hygiene were assessed using basic dental instruments, in artificial light, in clinical settings of the Department of Social Dentistry and Prophylaxis, Medical University of Białystok.**

Results and conclusions: **The record analysis showed a very high caries frequency index and a low treatment index. However, proper oral hygiene was observed, which may indicate greater health-promoting awareness among future dentists. Poor dentition status found in the study group of dental students may be due to neglect of oral hygiene, prophylaxis and lack of systematic dental control in the earlier age periods.**

Key worlds: dentition status, oral hygiene, dental students.

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Introduction

Caries, a social ailment, is one of the diseases of civilization of the 20th century. In Poland, the incidence rate of caries is very high both in the young and adults [1]. The major etiological factors of caries are: improper oral hygiene, diet based on carbohydrate-rich and highly processed food products, neglect of prophylaxis and dental check-up. In adolescents, additional cariogenic factors include eating snacks between meals, excessive consumption of sweets of sticky consistency and sparkling drinks. As data concerning the oral status of adolescents that reach us from different sources are alarming we decided to perform clinical dental examinations of the oral cavity among the first year dental students of the Faculty of Medicine, Medical University of Białystok in the year 2004/2005.

Aim

The aim of the study was to assess dental status and oral hygiene of the first year dental students, Medical University of Białystok, through the analysis of the chosen caries and dental plaque indices.

Material and methods

The study was based on the medical records filed by the first year students of the Division of Dentistry, Faculty of Medicine, Medical University of Białystok, during Social Dentistry classes in the academic year 2004/2005. The study group consisted of 70 first year dentistry students, including 50 women and 20 men, aged 19-23 years. Dentition status and oral hygiene were assessed using basic dental instruments, in artificial light, in clinical settings of the Department of Social Dentistry and Prophylaxis, Medical University of Białystok. The results were recorded in the charts specially designed for epidemiological studies according to WHO [2].

Clinical records were used to calculate the following indices:

Table 1. **Assessment of dentition status and oral hygiene index**

	DMFd	DMFs	SiC	TI*	OHI-S*
Women	11.72 ± 1.61	15.90 ± 2.18	16.73 ± 1.70	0.77 ± 0.22	0.54 ± 0.45
Men	12.40 ± 2.58	16.84 ± 3.51	17.67 ± 2.94	0.64 ± 0.24	0.86 ± 0.58
Total	11.91 ± 1.39	16.17 ± 1.89	17.00 ± 2.09	0.73 ± 0.24	0.63 ± 0.51

* statistically significant result ($p<0.05$)

- the dental caries frequency index (CF), being the percentage of people affected by caries,
- the mean DMF score expressed as the sum of DMF values, where D was the sum of teeth with active decay, M – teeth missing due to caries and periodontal disorders, F – filled teeth divided by the number of study subjects,
- the dental decay intensity index – DMFd, i.e. the quotient in which the sum of DMFd values for carious teeth was divided by the number of people affected by caries,
- the surface decay intensity index – DMFs, i.e. the quotient in which the sum of DMF scores for the surfaces affected by caries was divided by the number of people affected,
- the significant index of caries – SiC, which is the arithmetic mean of DMFd for 1/3 of the population with the highest DMFd scores,
- the treatment index – TI, i.e. the number of filled teeth to the number of carious teeth plus filled teeth.

Oral hygiene status was assessed by means of OHI-S, using dental plaque liquid dye (Butler) according to the producer's instructions. Soft and mineralized deposits were assessed on 6 surfaces of 6 teeth: buccal (16 and 26), lingual (36 and 46), labial (11) and lingual (31). The following criteria were applied: 0 – lack of colour, 1 – colour up to 1/3 of tooth crown, 2 – colour up to 2/3 of the crown and 3 – higher than 2/3 of the crown.

Results

The results were subjected to statistical analysis using Mann-Whitney test and presented in tables and diagrams. Differences were considered statistically significant for $p<0.05$ [3].

As the caries frequency index in the study group was 100%, the mean DMF score was equal to DMFd.

The overall DMFd index was 11.91, being lower for women (11.72) as compared to men (12.40), but the difference was statistically insignificant.

The overall DMFs index was 16.17, being statistically insignificantly lower in women (15.90) than in men (16.84).

The SiC showed a slight polarisation of caries. Overall, SiC value was 17.00, being lower in women (16.73) than in men (17.67), but with no statistically significant differences.

The analysis showed distinct differences in the treatment index between women and men. In women, the index was 0.77, in men - 0.64 and overall – 0. 73 at $p>0.05$.

The oral hygiene index OHI-S also revealed considerable gender-dependent differences, which was confirmed statistically. OHI-S was much lower in women (0.54) as compared to men (0.86), with 0.63 for the whole study group, $p<0.05$.

Discussion

A comparison of the findings obtained from similar studies conducted among students of dentistry has revealed a high level of caries frequency index being maintained for a few years. Kierklo, studying a group of third, fourth and fifth year dentistry students, Medical University of Białystok, in the year 1995, found the mean DMF score to reach 14.6 overall, with women being more affected by caries (15.4) as compared to men (12.2) [4]. In a study conducted by Wawrzyn-Sobczak and Stokowska, the CF index among first and second year dental students and fourth year medical students, Medical University of Białystok, was 97% in the year 2001/2002. The mean DMF score for the whole study population was 12.8, caries intensity index was 14.28, SiC – 19.9, and treatment index – 0.74 [5].

The study results obtained in our region in the previous years [6,7] show a significant drop in DMF score as compared to our findings.

Oral health targets have been designed for Poland according to the WHO criteria, stating that 90% of 18-year-old adolescents will have complete dentition by the year 2015. This requirement has been satisfied in our students [8].

Conclusions

Summing up, the present record analysis showed a very high caries frequency index and a low treatment index. However, proper oral hygiene was observed, which may indicate greater health-promoting awareness among future dentists. Poor dentition status found in the study group of dental students may be due to neglect of oral hygiene, prophylaxis and lack of systematic dental control in the earlier age periods.

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Prosthetic status and needs of HIV positive subjects

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Abstract

Purpose: **The aim of this study is an evaluation of existing dentition reconstructions in HIV-infected patients and definition of prosthetic needs of the examined population.**

Material and methods: **We examined 49 HIV-infected subjects (19-52 years of age) and 49 non-infected patients as the control group. Dental services were evaluated using treatment structure. The analysis of teeth loss was performed by using index created by Rogowiec. The area of prosthetic treatment was also defined. Aquired data were analyzed in examined populations regarding infection's duration time.**

Results: **Analysis of Rogowiec index values showed heavy losses in all anatomic groups of teeth and treatment structure index in the group of HIV infected subjects reached value 71.27%. The percentage of infected patients using prosthetic dentures was two times higher than in control group. In mandible, this difference was more significant. As the HIV infection's duration time increased, the percentage of subjets with prosthetic dentures in both dental arches also increased. Reconstruction of maxilla's dentition was necessary in 38.78% of HIV(+) subjects. In infected group, the necessity of reconstruction of teeth loss in lower dental arch reached 46.94%. As the infection's duration time increased, prosthetic needs of upper dental arch slightly decreased and needs of lower dental arch increased.**

Conclusions:

1. Using only emergency dental aid by HIV infected people results in significant loss of dentitnion.

2. Extraction domination over conservative reconstrucions in dental treatment, despite of young age of examined subjests, leads to damage of mastication organ.

3. The teeth loss in subjects infected for a longer period of time, results in increased need of prosthetic treatment.

Key words: HIV infection, prosthetic status, prosthetic needs.

Introduction

Prosthetic treatment of oral cavity is carried in order to improve aesthetics and reinstate lost mastication functions. All these premises are to improve quality of patient's life. Increasing loss of dentition is causing a necessity of mastication organ's rehabilitation by using dentures.

Oral cavity is mainly a preliminary segment of an alimentary tract and a significant dentition loss disturbs mastication functions [1]. The consequences of lack of dentures can be significant changes in an alimentary tract such as: chronic gastritis or more frequent and more intense *Helicobacter pylori* infections [1].

Aesthetic look of a patient can also have a significant meaning in a social life. HIV-infected patients are mainly young persons, under 30 whose dentition status is not satisfying. Significant dentition loss, also in frontal segment, is a consequence of an extreme life style. The main part of HIV-infected subjects are people, who are or who were addicted to narcotics [2]. Dentition lacks are often an obstacle to young people applying for a profitable job or trying to undertake social functions. They also have an impact on a low self-esteem of HIV-infected subjects.

AIDS symptoms occurs later due to contemporary High Antyretroviral Therapy, what lenghtens life time of a HIV-infected person. This is a premise of increasing meaning of restoring aesthetic and masticatory functions of masticatory organ in HIV-infected patients group.

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Table 1. **Tooth loss in examined populations according to infection's duration time**

Examined group	Number of subjects	Molars/pre-molars	Canines	Incisors
Infected <4 years (I)	30	4.90	0.40	0.73
Infected >4 years (II)	19	8.68	1.26	2.95
Infected in general (A)	49	6.45	0.73	1.55
Non-infected (B)	49	2.45	0.06	0.20
Statistical analysis – $p<0.05$ for groups		A and B	A and B	A and B
		I and II	I and II	I and II

Table 2. **Prosthetic status in HIV-infected subjects in regard to time of infection**

Examined group	Number of subjects	Maxilla								Mandible							
		Bridge		Bridge and removable denture		Removable denture		Total		Bridge		Bridge and removable denture		Removable denture		Total	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Infected <4 years (I)	30	0	0	1	3.33	3	10.00	4	13.33	0	0	1	3.33	0	0	1	3.33
Infected >4 years (II)	19	0	0	2	10.53	4	21.05	6	31.58	0	0	1	5.26	3	15.79	4	21.05
Infected (A)	49	0	0	3	6.12	7	14.29	10	20.41	0	0	2	4.08	3	6.12	5	10.20
Non-infected (B)	49	1	2.04	0	0	4	8.16	5	10.20	0	0	0	0	1	2.04	1	2.04

The aim of this study is an evaluation of existing dentition reconstructions in HIV-infected patients and definition of prosthetic needs of the examined population.

Material and methods

49 HIV-infected subjects were examined. They were patients of Department of Observation and Infection Medical University of Białystok, including 12 females and 37 males, 19-52 years of age (the average age – 30.65). 67.4% of HIV-infected subjects were intravenous drug users. The control group consisted of equal number of non-infected subjects, who were the same age and sex as the HIV(+) population.

Oral examination was conducted at artificial light using basic dental equipment according to WHO criteria from "Oral Health Surveys Basic Methods" [3]. The results were stored on examination cards, adjusted to study needs.

Aquired information on dentition status allowed to analyse existing dental services. Dental services were evaluated using treatment structure index [3,4]. Treatment structure index [4] is expressed by division of percentage of removed teeth (Mx100) to general number of effective services (M+F).

The analysis of teeth loss was performed by using index created by Rogowiec [5]. The area of prosthetic treatment was also defined.

Aquired data were analyzed in examined populations regarding infection's duration time. The time, from diagnosing the infection to examination time was considered as the infection's duration time. Subjects were divided into two groups according to infection's duration time:

- I group – subjects, whose infection's duration time was shorter than 4 years – 30 subjects,
- II group – subjects infected for more than 4 years – 19 subjects.

The aquired results were analyzed statistically. Hypotheses were verified by using t – student test or U Mann-Whitney's test and rejected if $p \leq 0.05$.

Results

Tab. 1 shows average values of tooth loss index according to Rogowiec. The data from the table shows higher general tooth loss in HIV(+) patients compared to non-infected subjects. The data concerns all teeth groups. In examined population, the highest tooth loss was found in side segment (molars and premolars). HIV(+) subjects lost an average 6.45 teeth, non-infected subjects lost only 2.45 teeth. Also in frontal segment, the tooth loss was higher in HIV-infected group. In the area of incisors, the loss of 1.55 teeth was found and in the area of canines – the loss of 0.73 tooth.

The tooth loss index in non-infected group was over 7 times lower for incisors and over 12 times lower for canines than in HIV(+) group. The data included in the analyzed table allowed examiners to analyse the tooth loss according to infection's duration time. The highes tooth loss was found in subjects infected for over 4 years (II group). Their average number of removed molars and premolars teeth reached 8.68, when as in I group, the number was 1.5 times lower – 4.90. As the infection's duration time increased, the frontal tooth loss index also increased. Subjects with shorter infection's duration time lost an avarege 0.73 incisor, where as subjects with longer infection's duration time lost 2.95 incisors. Similar relations were observed according to canines, which loss increased from 0.40 tooth in I group to 1.26 teeth in II group. The statistical analysis revealed significantly greater tooth loss in patients with longer infection's duration time in comparison with patients who were infected for a shorter period of time. That included the tooth loss in frontal and side segment.

The prosthetic status of examined populations according to infection's duration time is showed in *Tab. 2*. The percentage of

Table 3. **Prosthetic needs in examined populations**

				Examined group			
				Infected <4 years (I)	Infected >4 years (II)	Infected (A)	Non-infected (B)
				Number of subjects			
				30	19	49	49
Maxilla	Requires a reconstruction of one missing tooth (bridge)	1	n	1	0	1	3
			%	3.33	0	2.04	6.12
	Requires a reconstruction of more than one missing tooth (bridge or partial denture)	2	n	10	6	16	12
			%	33.33	31.58	32.65	24.49
	Requires a combination (bridge and partial denture)	3	n	1	0	1	0
			%	3.33	0	2.04	0
	Requires a complete denture	4	n	0	1	1	0
			%	0	5.26	2.04	0
	Total		n	12	7	19	15
			%	40.00	36.84	38.78	30.61
Mandible	Requires a reconstruction of one missing tooth (bridge)	1		2	2	4	1
				6.67	10.53	8.16	2.04
	Requires a reconstruction of more than one missing tooth (bridge or partial denture)	2		9	7	17	19
				30.00	36.84	34.69	38.78
	Requires a combination (bridge and partial denture)	3		1	0	1	0
				3.33	0	2.04	0
	Requires a complete denture	4		0	1	1	0
				0	5.26	2.04	0
	Total			12	10	23	20
				40.00	52.63	46.94	40.62

infected patients using dentures (20.41%) was two times higher than in control group (10.20%). In mandible, this difference was more significant, because dentures were present in 10.20% of infected patients and in 2.04% of non-infected subjets. HIV--infected subjects had mainly removable dentures. The presence of removable dentures was observed in maxilla in 14.29% of HIV-infected and in mandible in 6.12% of HIV positive subjects. Combination of bridge and partial denture was observed in maxilla in 6.12% of HIV positive subjects and in mandible in 4.08% of HIV-infected. As the HIV-infection's duration time increased, the percentage of subjets with dentures in both dental arches also increased.

Prosthetic needs of examined groups are showed in *Tab. 3*. Reconstruction of maxilla's dentition was necessary in 38.78% of HIV(+) subjects and in 30.61% of non-infected subjects. In infected group, the necessity of reconstruction of teeth loss in lower dental arch was higher (46.94%) compared to the control group (40.82%). As the infection's duration time increased, prosthetic needs of upper dental arch slightly decreased and needs of lower dental arch increased. The observation indicates that only persons with longer infection's duration time need to have complete denture of maxilla (5.26%) and mandible (5.26%) made.

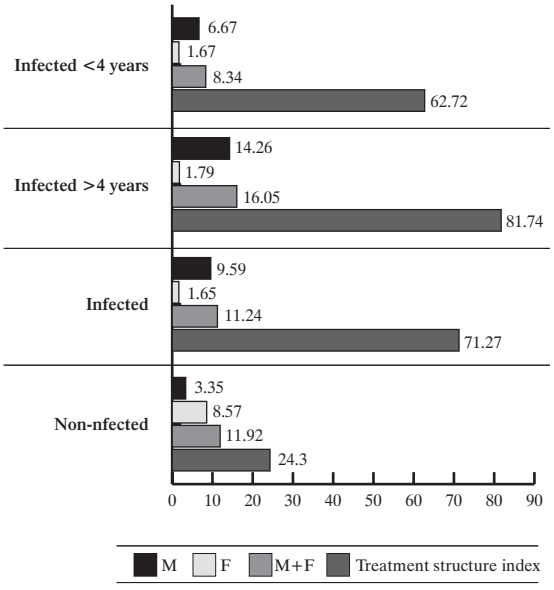
Treatment structure index, which enables the evaluation of relations between treatments provided in the conservative restorations and extractions area, in the group of HIV-infected subjects reached value 71.27%, where as in group with shorter infection's time it was 62.72% and as the infection's duration time increased, it increased to 81.74%. The increase of treatment structure index value with infection's duration time give evidence about increasing advantage of removed teeth to provided effective dentistic treatments in general. Clear, statistically significant differences in treatment structure were observed between infected group and control group. Values of treatment structure index in both infected populations (groups I and II) also differred statistically significantly (*Fig. 1*).

Results and discussion

HIV-infected subjects made a small percentage of people inhabiting Podlasie province. With regard to progressive increase of infected people, their dentition status and dental needs is becoming a problem, which needs to be solved as soon as possible. It is assumed, that 70-90% of HIV-infected people are intravenous drug users [6-8]. Bruziewicz-Mikłaszewska et al. [6] proved, that tooth ache is the main cause of drug users to visit dental practice, but then it is too late for conservative restorations and a tooth has to be removed.

Patients examined by me, are mainly drug addicts, who were infected with HIV during intravenous drug application. That is

Figure 1. **Treatment structure index in dependance on HIV infection's duration time**



why intensification of carries process needs to be connected to extreme life style, insufficient oral cavity hygene and stopping visiting to dental practise [6]. Dentition aesthetic disturbances in frontal segment also discourage HIV-infected people to visit dental practise, what is proved by significant number of lost teeth in frontal segment. Different results were presented by Matee et al. [9] when evaluating dentition status in HIV-infected people in Tanzania. In their opinion, there are no statistically significant differences in dentition status between HIV-infected and non-infected subjects.

The financial status of my examined patients was also important. More than half of them have never worked and has been supported only means from social welfare. In the population examined by Matee et al. [9], sexual intercourses were the main way of virus transmission, what did not have influence on life style or degradation of social and financial conditions in the way, it could be observed in drug addicts.

The result of avoiding visiting dental practice was the increase of teeth loss in persons living with HIV. Analysis of Rogowiec index values showed heavy losses in all anatomic groups of teeth. One needs to emphasize the high tooth loss index in frontal segment in HIV(+) persons, who had about seven times more incisors and twelve times more canines removed than in control group. Obtained results are confirmed by Szymaniak et al. studies [10]. Content analysis with infection's duration time taken under consideration, showed statistically significant lesser teeth loss in persons who had been infected for a shorter period of time. It has been documented in all teeth groups. Therefore my study confirmed the opinion, that a degree of dentition erosion is in proportion to HIV infection's duration time.

Basing on analysis of treatment structure index value, I evaluated the range of existing dental care. The index, in the HIV-infected group, turns out to be very disadvantageous. The analysis of treatment structure index shows that 71.27% of dental treatments on HIV-infected patients are teeth extractions. This disadvantageous structure has been documented by other authors [8,10], who emphasize the high number of extractions, which exceeds the number of conservative restoration.

The time of infection's duration significantly changes the value of treatment structure index, what is manifested by increase of the number of extracted teeth from 6.67 in the group of subjects with shorter infection time, to 14.26 in the group of subjects with longer infection time.

The tooth loss requires a prosthetic treatment. Dentures are used more frequently by infected persons than by non-infected. Disease's duration time of infected people had impact on the increase of the percentage of people using dentures. As far as prosthetic treatment is concerned, the needs for treatment are greater in HIV(+) group than in control group and increased in proportion to infection's duration time. Bruziewicz-Miklaszewska et al. [6] affirmed, that in spite of high percentage of HIV-infected people using dentures, there is still a significant group of patients with large, not reconstructed teeth loss. This opinion has been proved in my study's results.

Conclusions

1. Using only emergency dental aid by HIV-infected people results in significant loss of dentitnion.
2. Extraction domination over conservative reconstrucions in dental treatment, despite of young age of examined subjects, leads to damage of masitcatory organ.
3. The teeth loss in subjects infected for a longer period of time, results in increased need of prosthetic treatment.

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Periodontal status and treatment needs in HIV-infected patients

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Abstract

Purpose: **The aim of this study is evaluation of periodontal status and definition of periodontal treatment needs in HIV infected patients.**

Material and methods: **We examined 49 HIV-infected subjects (19-52 years of age) and 49 non-infected patients as the control group. Periodontal status and treatment needs were evaluated by using CPITN – Community Periodontal Index and Treatment Needs. Aquired data were analyzed in examined populations regarding infection's duration time and in dependance on absolute number of CD4 lymphocytes in μl of plasma, dividing patients according to criterion of HIV infection classification after CDC (Centers for Diseases Control and Prevention).**

Results: **More advanced changes in the paradontium were observed mostly in examined HIV infected subjets. As HIV infection time proceeds, the periodontal status of examined patients impairs, what is manifested by the decrease of the number of sextants with the intact paradontium and the increase of the number of sextants excluded from the research. There was no significant relation found between periodontal status evaluated with CPITN and the immunity status of examined subjects. 26.5% of HIV infected subjects needed the complex therapy. As the immunity decreased, the number of patients qualified to the complex treatment increased, and the number of HIV(+) patients with no need of therapy decreased.**

Conclussions:

1. As the infection duration time proceeds, the periodontal status in HIV-infected patients impairs.

2. Deterioration of health status, expreesed with decrease of absolute number of CD4 lymphocytes is accompanied by intensification of pathological periodontal changes.

3. HIV infected persons are group with high periodontal needs and require intensive periodontal care.

Key words: HIV infection, CPITN, periodontal status, periodontal needs.

Introduction

A cell which is sensitive to infection with Human Immunodeficiency virus is called permisive, which means a target cell [1]. A kind of infected cell is a fundamental issue in virus infection. Presence of an adequate receptor on cytomembrane's surface is a condidtion allowing virus to penetrate a cell. The best known receptor for HIV is a CD4 particle. It is a phenotype feature of mature T lymphocyte, which is part of subpopulation of helper T4 lymphocytes or CD4 cells [1,2]. Although infection and replication of HIV concerns many types of cells, it is organism pule of macrophages and monocytes which seems to be particulary important reservoir of virus, regarding to migtation abilities, positioning in the organism and large sumaric mass. Local tissue damage in HIV infection process is conneted to macrophages tissue population [3] and disability of T lymphocytes' funtions [1]. Damage of T lymphocytes population influences the process of production of specific antibodies by B lymphocytes, it also influences cytokines secrete which activate cell immunity [4]. Local immunity distortion of this type in HIV infected patients can lead to inflamation changes in parodontium.

Periodontal problems are frequent and mostly first symptoms of retroviral infection in HIV infected subjects [5]. Systematizing of periodontal diseases in HIV infection turned out to be difficult because of differences in personal immunity for viral infection. Attempt of systematics unification of periodontal diseases in HIV infected persons was undertaken by Holmstrup

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Table 1. **Periodontical status since infection**

Absolute lymphocyte CD_4/μl number	Number of subjects	CPI-0 x	CPI-1 x	CPI-2 x	CPI-3 x	CPI-4 x	CPI-X x
Infected <4 years (I)	30	1.03	2.63	1.17	0.27	0.00	0.90
Infected >4 years (II)	19	0.74	1.95	1.05	0.68	0.05	1.53
Infected in total (A)	49	0.88	2.29	1.20	0.45	0.04	1.14
Non-infected (B)	49	2.92	2.06	0.78	0.00	0.00	0.24
Statistical analysis: p<0.05 for groups		A and B	-	A and B	A and B	-	A and B

Table 2. **CPITN acording to CD_4 cells count**

Absolute lymphocyte CD_4/μl number	Number of subjects	CPI-0 x	CPI-1 x	CPI-2 X	CPI-3 x	CPI-4 x	CPI-X x
>500/μl (1)	8	1.50	2.75	1.25	0.50	0.00	0.00
200-499/μl (2)	27	0.96	2.48	1.07	0.48	0.00	1.00
<200/μl (3)	14	0.50	1.64	1.21	0.29	0.14	2.21
Statistical analysis: p<0.05 for groups		-	-	-	-	-	1 and 2 2 and 3 1 and 3

i Westergaard [5], who suggest, basing on Riley et al. research [cite after 5], that in HIV-infected persons, besides inflamations typical for HIV infection, also typical forms of gingivitis and periodontitis occur. According to data from references, HIV(+) persons in contrary to non-infected subjects often suffer from linear gingivitis and/or linear gingival erythema and necrotizing ulcerative periodontitis [6,7].

The aim of this study is evaluation of periodontal status and definition of periodontal treatment needs in HIV-infected patients.

Material and methods

49 HIV-infected subjects were examined. They were patients of Department of Observation and Infection Medical University of Białystok, including 12 females and 37 males, 19-52 years of age (the average age – 30.65). The control group consisted of equal number of non-infected subjects, who were the same age and sex as the HIV(+) population. Oral examination was conducted at artificial light using basic dental equipment according to WHO criteria from "Oral Health Surveys Basic Methods" [8].

Periodontal status and treatment needs were evaluated by using CPITN – Community Periodontal Index and Treatment Needs [8,9]. Aquired data were analyzed in examined populations regarding infection's duration time. The time, from diagnosing the infection to examination time was considered as the infection's duration time. Subjects were divided into two groups according to infection's duration time:

- I group – subjects, whose infection's duration time was shorter than 4 years – 30 subjects
- II group – subjects infected for more than 4 years – 19 subjects.

Results were considered in dependance on absolute number of CD4 lymphocytes in μl of plasma, dividing patients according to criterion of HIV infection classification after CDC (Centers for Diseases Control and Prevention). Three ranges of laboratory values were considered [10]:

- number of CD4 cells over 500/μl (8 subjects),
- number of CD4 cells from 200/μl do 499/μl (27 subjects),
- number of CD4 cells under 200/μl (14 subjects).

The results were analyzed statistically using Statistica 5.0 software. The differences, for which values of "significance" p are lower or equal to 0.05 ($p \leq 0.05$), were considered significant.

Results

The analysis of the periodontal status in examined populations was conducted basing on community periodontal index and treatment needs (*Tab. 1*). Most of all healthy sextants qualified as CPI-0 were found in the non-infected group (2.92). Significantly less, 0.88 of healthy sextant was found in the HIV--infected group. In both populations the number of sextants with the gingivorrhoea was similar (group A – 2.29, group B – 2.06). The average number of sextants with the code CPI-2, showing on the presence of the dental calculus, amounted, in infected subjects – 1.20, while in non-infected subjects – 0.78, this difference was statistically significant. More advanced changes, manifesting themselfs with the presence of periodontal pockets with the depth exceeding 4 millimetres (CPI-3 and CPI-4) were ascertained only in the examined HIV(+) group. When conducting the CPITN evaluation, average 0.24 of sextant in non-infected subjects and 1.14 in infected subjects were excluded

Table 3. **Periodontal treatment needs of the examined populations with time factor taken under consideration**

Infection duration time	Number of subjects	Periodontal treatment needs TN							
		Code 0		Code 1		Code 2		Code 3	
		n	%	n	%	n	%	n	%
Infected <4 years (I)	30	1	3.33	9	30.00	15	50.00	5	16.67
Infected >4 years (II)	19	2	10.5	2	10.5	7	36.8	8	42.1
Infected in total (A)	49	3	6.1	11	22.5	22	44.9	13	26.5
Non-infected (B)	49	9	18.4	19	38.8	23	46.9	0	0
Statistical analysis: p<0.05 for groups		A and B		A and B		-		A and B	

Table 4. **Periodontal treatment needs and a CD_4 cell count**

Absolute lymphocyte CD_4/µl number		Number of subjects	Periodontal treatment needs TN							
			Code 0		Code 1		Code 2		Code 3	
			n	%	n	%	n	%	N	%
Infected	CD_4 >500/µl (1)	8	21	12.5	3	37.5	2	25	2	25
	CD_4 200-499/µl (2)	27	2	7.7	5	19.2	14	53.8	5	19.2
	CD_4 <200/µl (3)	14	0	0	2	15.4	6	46.2	5	38.5
Statistical analysis: p<0.05 for groups			-		-		-		-	

from the study, because of loss of indexed teeth. The infection's duration time did not influence significantly on the periodontal state. Although, as the infection's duration time proceeded, the decrease of the average number of sextants with low numbered codes i.e. with the healthy paradontium, with the gingivorrhoea and the dental calculus and the increase of the number of sextants with deep periodontal pockets and the number of sextants excluded from the study were observed, observed changes were statistically insignificant.

There was no significant relation found between periodontal status evaluated with CPITN and the immunological status of examined infected subjects. Instead, the negative correlation between advance of the disease and the average number of sextants (CPI-X) excluded from the study, was observed. Patients with the high immunity had no sextants excluded from the research. In infected subjects with the number of CD4 cells amounted 200-499/µl, an average 1.00 of sextant was excluded, and in subjects with the lower number of CD4 cells 2,21 of sextants were excluded. Statistically significant differences were observed between groups 1 and 3, 1 and 2, 2 and 3 (*Tab. 2*).

Periodontal treatment needs of examined populations with the infection duration time taken under consideration were presented in *Tab. 3*. The treatment of the paradontium (code 0) was not needed by 17.6% of subjects from the control group and by 6.1% of examined infected patients. Instruction of the oral hygiene (code 1) was needed by 16.9% more of subjects from the B group than from the A group, what was statistically significant. The similar number of subjects from both examined populations demanded the instruction of the oral hygiene, scaling and removal of overhangs of fillings (A group – 46.9%, B group – 45.1%). The complex periodontal treatment (code 3) was needed only by infected patients. The HIV infection duration time did not influence periodontal treatment needs. As the infection duration time proceeded, the percentage of subjets who did need treatment increased about 8.5%, and the percentage of those in need of oral hygiene instruction decreased about 24.1%. Needs with code 2 were similar in both groups: shorter infected (50%) and longer infected (47.1%). The complex treatment was needed by 16.7% of patients with shorter infection time and about 18.6% more of those with longer infection time.

The *Tab. 4* presents periodontal treatment needs in depence on the immunity status of examined subjects. Although there were no statistical relations, it was observed that the decrease of the CD4 lymphocytes count in one microlitre of plasma was accompanied by the percentage of patients who did not need periodontal treatment (from 12.5% in the group with the high CD4 cell level to 0% in subjets with low values of CD4 lymphocytes). The similar correlation was observed in the case of treatment needs with code 1 (oral hygiene instruction). Most of patients in need of oral hygiene instruction, scalling and the removal of fillings overhangs and complex periodontal treatment was in the group with the number of lymphocytes CD4 200-499/µl.

Results and discussion

Conclusions from our study are the number of sextants with the healthy paradontium in the examined HIV infected group was over three times lower, and the number of sextants excluded from the research about five times higher than in the control group. Similar results were obtained by Stangiewicz [11], who ascertained that in HIV infected subjects examined by her there was average one sextant with code 0, which was the proof of the lack of changes in the paradontium. A repeating manifestation in all examined subjects was bleeding caused by probing, which in our research referred to 2.29 of sextants in HIV(+) patients and 2.06 of sextants in healthy subjects. More advanced changes

in the paradontium were observed mostly in examined HIV infected subjets. The presence of the dental calculus or other retention-traumatic factors was observed twice more often in infected group, where as periodontal pockets with depth exceeding 4 mm's were observed only in examined HIV infected group. Comparable relations were showed by Stangiewicz [11], however, her results indicate the higher number of sextants with the dental calculus and both shallow and deep periodontal pockets.

As HIV infection time proceeds, the periodontal status of examined patients impairs, what is manifested by the decrease of the number of sextants with the intact paradontium and the increase of the number of sextants excluded from the research. It is the evidence, that also the the number of sextants with the presence of moderate and deep periodontal pockets increases.

There are also very few infomation about the connection between the immunity status of examined subjects and the state of their paradontium. In the available literature, this problem was examined only by Barr et al. [12]. Results of their research prove the slight influence of the immunity status on the advance of periodontal changes. The data obtained by us confirmed these relations, because there was no significant relation found between periodontal status evaluated with CPITN and the immunity status of examined subjects.

HIV infected patients are the group that needs special periodontal care. Many authors perceive periodontal diseases as the oral cavity changes, which most often accompany the HIV infection [12,13]. The periodontal treatment was not needed only by 6.1% of examined infected subjects, and 26.5% of them needed the complex therapy. As the immunity decreased, the number of patients qualified to the complex treatment increased, and the number of HIV(+) patients with no need of therapy decreased.

Taking the fact that the most HIV infected patients are drug addicts, under considetation, it can be accepted that the intensification of disease processes within the oral cavity is the result of the social and psychical disintegration and the discontinuation of the oral hygiene [14,15]. In the course of using opiates, the hierarchy of needs and values in those examined patients changes. Both fear of dental procedures and fear of lack of acceptation from the doctor are dominating feelings of drug users who ceased using narcotics. It causes the postponement of visits, what leads to further, considerable destruction of the dentition and in consequence, to the loss of teeth.

Conclusions

1. As the infection duration time proceeds, the periodontal status in HIV infected patients impairs.
2. Deterioration of health status, expreesed with decrease of absolute number of CD4 lymphocytes is accompanied by intensification of pathological periodontal changes.
3. HIV infected persons are group with high periodontal needs and require intensive periodontal care.

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Comparative analysis of the effect of preparations used in professional fluoride prophylaxis on the chosen parameters of human saliva

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Abstract

Regular supply of fluoride ions to the oral environment is one of the prophylactic actions against dental caries. Fluorides, whose exogenous action combines with saliva properties, condition the anticariogenic effect. Fluoride ions exhibit high chemical activity, can alter the oral environment parameters and inhibit the activity of enzymes.

Purpose: In the current study, the effect of fluoride preparations used in professional caries prophylaxis on chosen saliva parameters was studied. The levels of pH and fluoride ions, and the activity of cathepsin D in human saliva were determined.

Material and methods: Material for analysis contained resting mixed saliva collected before and 1, 4 and 24 hours after the application of Duraphat, Elmex Gel, Fluor Protector, Fluormex Gel and Fluoro-Gel.

Results: The fluoride-containing preparations inhibited the activity of cathepsin D in the way depending on the time that had passed since the application and altered the pH level of human saliva.

Key words: fluorides, saliva, cathepsin D.

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Introduction

Due to a number of functions, saliva plays a significant role both in physiology and pathology of the oral cavity. While the properties of saliva affect the course of various processes in the mouth, many factors can influence and alter the saliva parameters.

Caries prevention involves the arrest of etiological agents of dental caries and through application of fluoride compounds resistance of dental hard tissues is increased. The inorganic and organic fluoride compounds are found in preparations used both in everyday oral hygiene procedures and in professional fluoridation.

The major cariostatic mechanism of fluoride compounds is based on local action. They inhibit enamel demineralization, improve remineralization and decrease the activity of dental plaque bacteria [1]. Enamel stability in the oral environment depends on the pH of saliva and dental plaque, and is related to the concentrations of calcium, phosphate and fluoride ions. Depending on the concentration of a topically applied fluoride compound, the reaction of fluoride ions with enamel can yield fluorohydroxyapatite (after low-dose fluoride preparation) or calcium fluoride (after professional fluoridation) [2]. Fluoride ions contained in these compounds show high biochemical activity and can thus alter the oral environment parameters. Cathepsin D (EC 3.4.23.5) is a lysosomal endopeptidase that splits peptide bonds formed by carboxy groups of hydrophobic amino acids. It is isolated from tissues and organs, its trace amounts being found in blood plasma and body fluids including saliva. Cathepsin D takes part in pathological processes associated with myocardial ischaemia, liver disorders, muscular dystrophy, inflammatory states of joints and gums. It is involved in degradation of lipoproteins, parathyroid hormone, thyreoglobulin and glucagon [2,3].

In the study, the effects of the preparations commonly used for professional exogenous fluoride prophylaxis on the chosen parameters of saliva were compared. Levels of saliva pH and F^-, and activity of cathepsin D were determined.

Table 1. **Mean values of the saliva parameters studied**

		Duraphat				Elmex Gel				Fluor Protector			
		Mean	N	SD	Mediana	Mean	N	SD	Mediana	Mean	N	SD	Mediana
pH	0 h	7.06	6	0.33	7.00	7.46	6	0.43	7.47	7.27	6	0.15	7.29
pH	1 h	7.41	6	0.19	7.41	7.65	6	0.35	7.65	7.49	6	0.18	7.45
pH	4 h	7.42	5	0.15	7.48	7.53	6	0.26	7.43	7.39	6	0.16	7.34
pH	24 h	7.09	6	0.29	7.05	7.39	6	0.29	7.31	7.45	6	0.21	7.49
F^-[mg/dm^3]	0 h	18.96	6	12.74	16.46	2.11	6	1.33	2.09	0.40	6	0.13	0.39
F^-[mg/dm^3]	1 h	50.63	6	27.23	50.45	3.91	6	2.57	4.11	0.48	6	0.14	0.42
F^-[mg/dm^3]	4 h	51.89	5	21.80	56.23	3.73	6	1.85	3.51	0.46	6	0.12	0.46
F^-[mg/dm^3]	24 h	22.99	6	10.13	21.17	2.41	6	0.90	2.02	0.53	6	0.13	0.55
KAT D [nmol/ml]	0 h	136.67	6	29.90	137.00	158.00	6	54.10	129.50	97.67	6	38.40	83.00
KAT D [nmol/ml]	1 h	71.83	6	51.50	69.50	129.83	6	62.40	116.50	79.17	6	43.88	74.00
KAT D [nmol/ml]	4 h	80.00	5	16.25	76.00	125.40	5	55.94	105.00	147.83	6	59.38	132.00
KAT D [nmol/ml]	24 h	102.83	6	41.14	91.00	156.17	6	72.83	150.00	146.67	6	59.70	138.50

		Fluormex Gel				Fluoro-Gel				Razem			
		Mean	N	SD	Mediana	Mean	N	SD	Mediana	Mean	N	SD	Mediana
pH	0 h	7.12	6	0.26	7.09	7.03	6	0.12	7.08	7.19	30	0.31	7.13
pH	1 h	7.52	6	0.33	7.56	7.13	6	0.12	7.19	7.44	30	0.29	7.39
pH	4 h	7.41	6	0.23	7.38	7.18	6	0.13	7.24	7.38	29	0.22	7.30
pH	24 h	7.29	5	0.30	7.26	7.17	6	0.14	7.16	7.28	29	0.27	7.23
F^-[mg/dm^3]	0 h	8.86	6	5.66	6.84	16.14	6	7.44	15.55	9.29	30	9.97	6.65
F^-[mg/dm^3]	1 h	15.22	6	8.11	15.01	32.16	6	22.84	29.71	20.48	30	24.33	10.49
F^-[mg/dm^3]	4 h	12.96	6	3.59	13.78	26.00	6	14.43	23.75	17.87	29	21.02	9.12
F^-[mg/dm^3]	24 h	10.99	5	4.23	9.66	17.55	6	7.39	17.78	10.89	29	10.49	9.12
KAT D [nmol/ml]	0 h	164.17	6	29.46	166.50	93.67	6	53.56	71.50	130.03	30	49.59	127.00
KAT D [nmol/ml]	1 h	133.00	6	23.55	128.00	70.67	6	44.01	51.00	96.90	30	52.17	95.50
KAT D [nmol/ml]	4 h	133.17	6	61.24	108.50	75.67	6	48.33	56.50	113.11	28	56.42	104.50
KAT D [nmol/ml]	24 h	158.80	5	27.94	158.00	106.33	6	91.55	73.50	133.31	29	64.15	134.00

Material and methods

Material for analysis contained mixed saliva collected directly to test-tubes (ZPS, Medlab), two hours after a meal, from 30 dentistry students aged 19-24 years (21 women and 9 men). Samples were obtained before (baseline measurement) and directly after a single application of fluoride preparation, as well as after 4 and 24 hours. The tubes containing saliva were immediately frozen at -18 to -24°C. After defrosting, saliva pH was measured by ionoselective fluoride electrode. The activity of cathepsin D was determined by adding 0.1 ml of 6% haemoglobin to 0.4 ml of saliva. The samples were incubated for four hours at 37°C. The reagents used had a pH 3.5. The reaction was discontinued by adding 0.5 ml of 10% TCA and then the samples were centrifuged 2 700 x g, for 30 min, at 4°C. Samples that precipitated at baseline measurement were referred to as control. Acid-soluble tyrosine was determined in the supernatant using the method of Folin and Ciocalteau in copper modification. The amount of released tyrosine was read from the calibration curve designed according to the model solutions of this amino acid [4].

The following preparations were used for contact fluoridation procedures: Fluor Protector, Elmex Gel, Fluormex Gel, Duraphat, Fluoro-Gel. Results were statistically analysed using the two-factor analysis of variance for repeatable measurements with Fisher NIR post hoc tests, using Statistica 6.0 programme, Statsoft. The accepted level of significance was 0.005.

Results

Tab. 1 presents mean values of the saliva parameters determined in the current study, taking into account type of fluoride preparation used and time that passed since fluoridation. Groups were characterized by calculating the mean, median and standard deviation.

Saliva pH values after contact fluoridation increased as compared to those before application. Type of preparation and time that passed since fluoridation had a statistically significant effect on pH. *Fig. 1* shows saliva pH values after application of the preparations used, being the highest for Elmex Gel. Statistically significant differences in the increase in saliva pH between the preparations were found between Elmex Gel and Fluoro-Gel, Elmex Gel and Duraphat, and Fluor Protector and Fluoro-Gel.

The pH value was found to depend on the time of measurement after fluoridation. *Fig. 2* presents pH values averaged for all the preparations according to the time point. The highest increase in pH was observed directly and 4 hours after fluoridation procedure as compared to the baseline one. After 24 hours, the pH dropped. The differences were statistically significant in comparison to those before fluoridation. No statistically significant differences were noted between pH value directly and 4 hours after fluoridation

A comparison of the pH values for the respective prepara-

Figure 1. **Mean pH values for the parameters studied, 95% confidence**

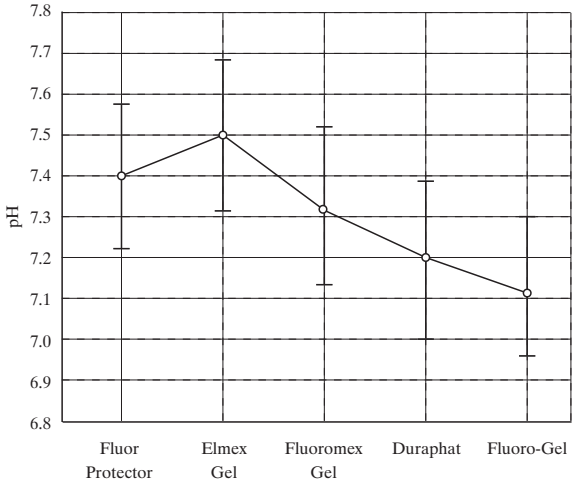


Figure 2. **Mean pH value for all preparations depending on time of measurement (95% confidence)**

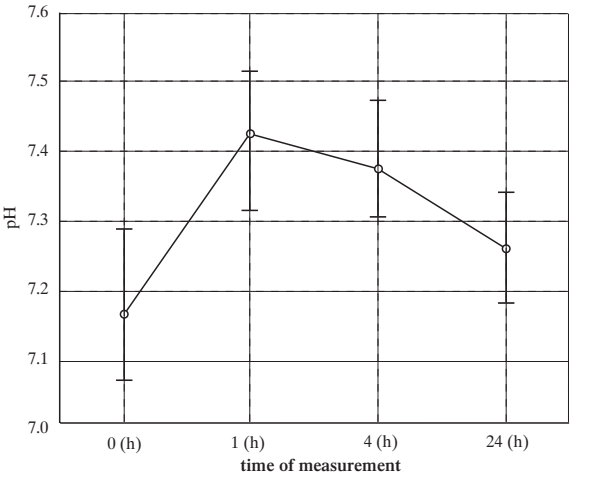


Figure 3. **Mean pH value for the respective preparation at a particular time point**

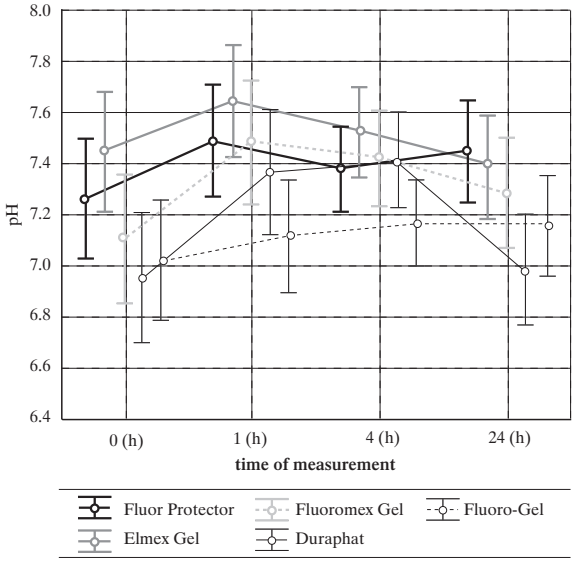
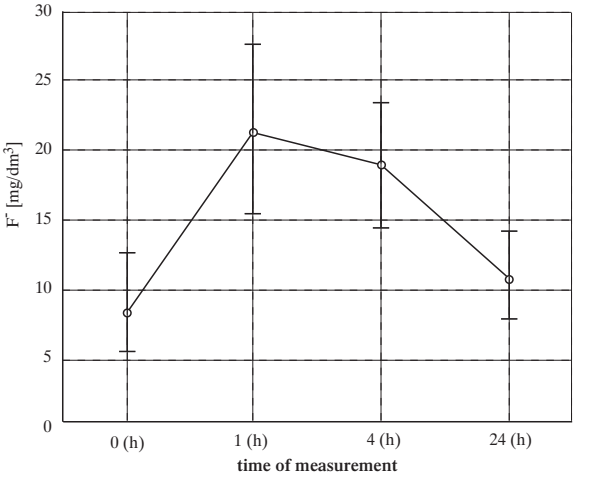


Figure 4. **Mean F^- values for the parameters studied, 95% confidence**



tion between various time points revealed a statistically significant increase for Fluor Protector at the time point directly after application as compared to baseline and between baseline and the level after 24 hours. The same tendency was noted when Elmex Gel, Fluormex Gel and Duraphat were applied. No statistically significant increase in saliva pH at all the time points was observed for Fluoro-Gel.

Fig. 3 demonstrates the mean pH value for the respective preparation at a particular point of measurement. A comparison between various preparations showed statistically significant differences only for Fluoro-Gel as compared to Elmex Gel directly after fluoridation. The level of fluoride ions measured before the application of fluoride preparations was found to undergo a marked increase directly after fluoridation, a slight decrease after 4 hours and a further drop after 24 hours, finally reaching a slightly higher level than before fluoridation (*Fig. 4*). However, the differences between the level of F^- before and 24 hours after application were not statistically significant. Analysis of variance indicates a statistically significant correlation of the level of F^- with type of fluoride preparation and time of measurement.

No statistically significant increase in saliva F^- was noted at any time points after application of Fluor Protector, Elmex Gel or Fluormex Gel. Duraphat caused the highest statistically significant rise in saliva F^- directly and 4 hours after fluoridation (*Fig. 5*). 24 hours after fluoridation, the level of F^- remained slightly but not statistically significantly elevated, as compared to that before fluoridation. However, drop in the level of F^- after 24 hours in comparison to examination directly and 4 hours after application was statistically significant. A similar distribution of F^- in saliva was observed after application of Fluoro-Gel (*Fig. 6*). No statistically significant differences were found between Elmex Gel and Fluormex Gel with regard to their effect on saliva fluoride ions. The increase in fluoride ions after application of Duraphat and Fluoro-Gel in comparison to

***Figure 5*. Mean F^- value for all preparations depending on time of measurement (95% confidence)**

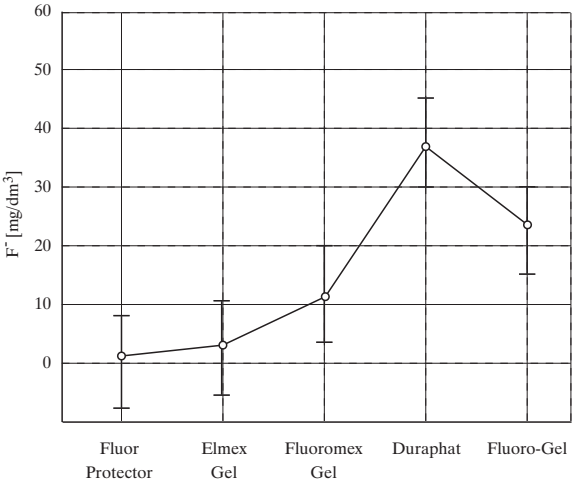


***Figure 6*. Mean F^- value for the respective preparation at a particular time of measurement**

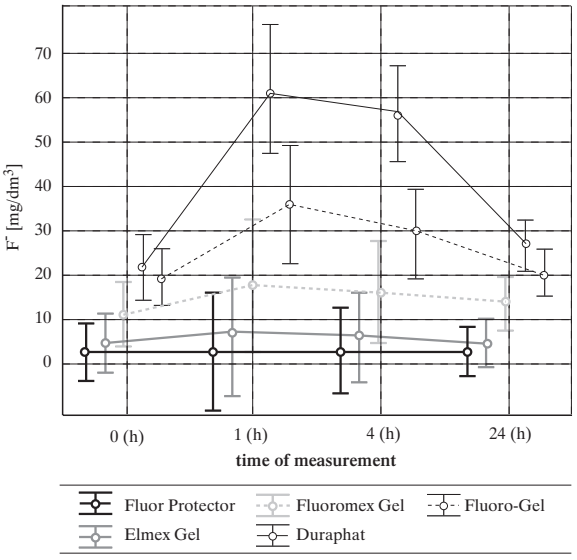


***Figure 7*. Mean cathepsin D level for all the preparations depending on time of measurement (95% confidence)**

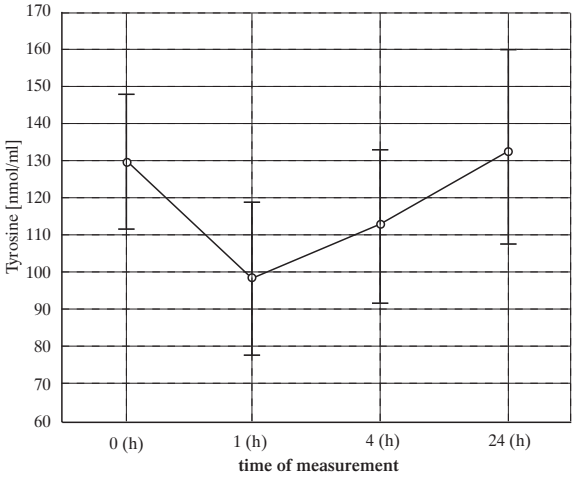


***Figure 8*. Mean cathepsin D level for the preparations studied, 95% confidence**

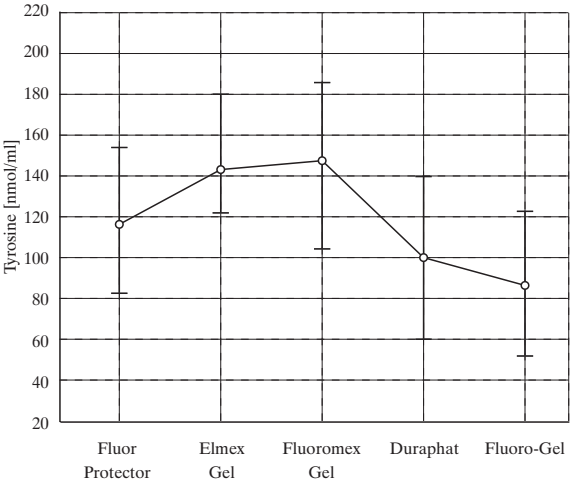
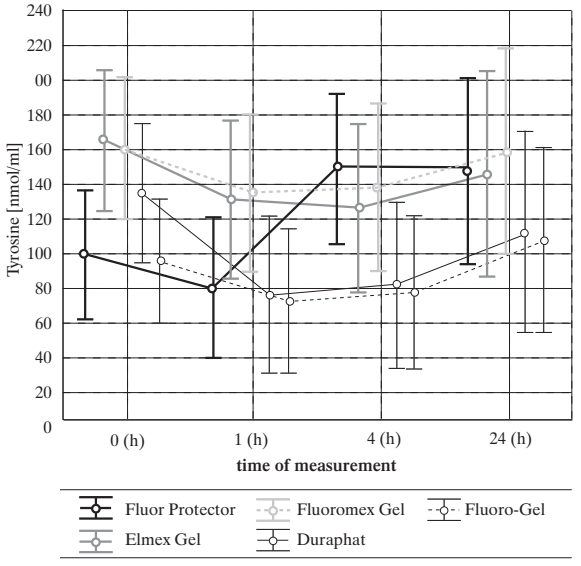


***Figure 9*. Mean level of cathepsin D for the respective preparation at a particular time point**



other preparations was statistically significant. A comparison of F^- level between various preparations at the respective time point revealed lack of statistically significant differences between Duraphat and Fluoro-Gel. The F^- values observed after application of the remaining preparations were lower as compared to Duraphat and Fluoro-Gel, the differences being statistically significant.

Measurements of saliva cathepsin D revealed its reduction directly after application of all the preparations studied (*Fig. 7, 9*). Differences in the effects between the respective preparations on cathepsin D in saliva were basically not statistically significant, and were only observed between Fluoro-Gel and Elmex Gel and Fluormex Gel (*Fig. 8*). The time point affected the level of cathepsin D and was a statistically significant factor. Statistically significant differences in the level of cathepsin D were found before and directly after fluoridation, when its level decreased, and between the examination directly

after and 24 hours after fluoridation, when its level increased (*Fig. 7*). The effects of various preparations on the level of cathepsin D at a particular time point showed no statistically significant differences.

Discussion

Fluoride compounds used for therapeutic reasons, thanks to their high chemical activity, can affect various biological systems. Affinity of fluoride ions for calcium and magnesium results in the effect on enzymatic activity. The compounds inhibit the action of oxidoreductases, transferases, hydrolases and the Krebs cycle enzymes [5-8]. The effect of fluorides on extracellular enzymes is also known. They inhibit, *in vitro* and *in vivo*, depending on their concentration and environmental pH, the activity of peroxidase and myeloperoxidase, the salivary antibacterial, non-immunological defence factors [9-11]. Endogenous and exogenous supplementation with fluoride compounds in caries prophylaxis is to ensure regular provision of fluoride ions to the oral cavity. The salivary level of fluoride ions increases on endogenous delivery or after topical application of fluoride preparations, but fluoride retention is unstable. In the current study, Duraphat and Fluoro-gel caused the most pronounced and statistically significant increase in the salivary F^- level immediately and 4 hours after application. This may be related to high fluoride content in these preparations and perhaps too low F^- availability to form calcium fluoride. The remaining preparations caused an increase in fluoride ions, which was, however, statistically insignificant. This would require further study.

In the current analysis, the pH value was elevated after contact fluoridation, being the highest immediately following the procedure and after 4 hours. The increase was statistically significant for all the preparations except for Fluoro-gel. Our results are consistent with those reported by other authors [12]. Dąbrowska et al. observed a slight increase in salivary pH after a single application of each of the preparations [13]. During saliva collection for analysis, the saliva secretion rate was found to increase directly after the use of prophylactic preparations (data not included in the report). Analysing the causes of the saliva pH increase even in the case of a slightly acidic pH (Elmex Gel), which promotes formation of calcium fluorides, a potential relationship between this increase and saliva secretion rate should be considered. Fluoridation can stimulate saliva secretion, resulting in elevated pH level. Engel-Brill et al. found significantly higher saliva secretion after brushing the teeth with an aminofluoride preparation in comparison to a monophosphate-containing preparation. The authors suggest increased saliva flow with an increase in the level of fluoride ions in aminofluorides [14].

Studies on the mechanism underlying the development of periodontal diseases have made an attempt to determine the role of proteolytic enzymes in the oral cavity. High activities of cathepsin B and L were noted in gingival tissue homogenates in periodontal patients [15-17]. Based on gingival biopsy, Jotterand and Cimasoni determined a statistically significant correlation between cathepsin D activity and gingivitis grade [18].

The study on the level of cathepsin D in the saliva showed its reduction directly after fluoridation, which was statistically significant. The inhibitory effect of oral hygiene preparations on proteolytic enzymes of the saliva was observed in vitro. A decrease was found in the activity of cathepsin D in human saliva in the presence of Blend-a-med toothpaste [19]. Dąbrowska et al. revealed a substantial reduction in cathepsin C activity in saliva following application of preparations containing amino fluorides [13].

Conclusion

In conclusion, it was found that professional and individual local fluoridation procedures caused a reduction in the activity of cathepsin D in human saliva and a transitory change in salivary pH.

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The evaluation of CPITN index among adults living in Podlasie region

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Abstract

The aim of this study was to evaluate the condition and treatment needs of the periodontium in adults living in Podlasie region. Checked population was divided into three groups: 18 year old, 35-44 and 65-74 year old. The assessment of the periodontium status was performed on the basis of CPITN index. The study showed that young people usually did not need any periodontal treatment. The predominating treatment need was removing of dental calculus, respectively 7.4% subjects aged 18, 62.5% of second group and 58.7% of the oldest one. 10% persons aged 35-44 and 6.9 % persons aged 65-74 required complex periodontal treatment. The number of excluded sextants grown with aged.

Key words: adult, CPITN, Podlasie.

Introduction

Periodontal diseases are chronic diseases that irreversibly destroy the supporting tissues of teeth. There are many studies showing that periodontal diseases are one of the main causes of tooth loss, especially among adults over 40 years of age [1,2]. The prevalence of periodontal problems as well as their severity increases with age. Some authors claim that periodontal status may also depend on sex, place of residence and socio-economic background [3,4].

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The purpose of this study was to evaluate the prevalence of periodontal diseases and periodontal treatment needs using Community Periodontal Index of Treatment Needs (CPITN) among adults living in region of Podlasie.

Material and methods

The examined population was divided into three groups: 166 people aged 18 years were qualified to group one, group two consisted of 40 patients aged from 35 to 44 years, and group three of 38 people aged 65-74. The subjects in every group were both sexes. The examination was carried out according to the guideline of The World's Health Organization [5] using an artificial light, a dental mirror and WHO periodontal probe (LM-Instruments Oy, Finland). The periodontal condition was evaluated by Community Periodontal Index of Treatment Needs (CPITN) [6,7]. The oral cavity was divided into 6 sextants: four consisting of molars and premolars, and two consisting of incisors and canines. There should be at least two teeth in each sextants, the single tooth was added to adjacent group. One tooth in each sexstant was examined for presence of gingival bleeding (code 1), dental calculus (code 2) and periodontal pocket (code 3 if its depth was 3.5-5.5 mm, and code 4 if it was 6 mm or deeper). Code 0 meant healthy periodontium. The examination was carried out on first upper and lower molars, and first right incisor in maxilla, and first left incisor in mandibula; if the tooth was not present in mouth the examiner chose another one from the sextant. On this basis the individual level of treatment needs (TN) was pointed out. TN 0 corresponded to healthy periodontium with no treatment needs. Patients with gingival bleeding needed oral hygiene instruction (TN 1), those with code 2 and 3 needed also scaling (TN 2). If there was at least one sextant with CPITN 4, the person was qualified for complex periodontal treatment.

Table 1. **Percentage of peolpe according to number of examined sexstants**

Group		Number of examined sextants					
		6	5	4	3	2	1
18	n	162	0	0	0	0	0
	%	100.0	0	0	0	0	0
35-44	n	23	5	5	3	0	4
	%	57.50	12.50	12.50	7.50	0	10.0
65-74	n	3	2	5	1	8	10
	%	10.40	6.90	17.20	3.40	27.60	34.50

Table 2. **Percentage of sextants according to CPITN code**

Group		Number of excluded sextants	Number of sextants with CIPTN code				
			0	1	2	3	4
18	n	0	956	2	8	6	0
	%	0	98.30	0.20	0.80	0.70	0
35-44	n	44	89	43	46	11	7
	%	18.30	37.10	17.90	19.20	4.60	2.90
65-74	n	98	22	17	23	9	5
	%	56.30	12.60	9.90	13.20	5.20	2.80

Results

All 18 year olds had teeth in every sextant – there were no excluded ones. The same status was represented by 57.5% people aged 35-44. Almost one out of 4 people from group three was endentulous, the were not taken into account when CPITN was evaluated. Only 10.4% of dentate elderly persons had teeth in every sextant. In this particular group the most common situation was the presence of teeth in one (34.5%) and two sextants (27.6%). The percentage of subjects according to number of examined sextants was presented in *Tab. 1*.

In the population of 18 year old total number of checked sextants was 972; 98.3% of them were healthy. In group two 18.3% sextants were excluded from the study, in the oldest group it was more than half of total number of sextants (56.3%). CPITN code 1 was revealed in 17.9% sextants as people were 35-44, and in 9.9% as they were over 65. Dental calculus was observed in one out of five sextants in group number two, and presence of periodontal diseases was revealed in 7.5% sextants: 4.6% with periodontal pockets 3.5-5.5 mm, and 2.9% with deeper ones. In the last group 13.2% of examined sextants presented with dental calculus, and the prevalence of periodontal pockets was similar to people in middle age. The results were shown in *Tab. 2*.

92% of young subjects had healthy periodontium (code 0), and there was no treatment needs for them (TN 0). Only one person suffered from gingival bleeding. There were 12 people in this age with TN 2 because of presence of dental calculus or periodontal pocket from 3.3 to 5.5 mm. The study did not reveal any young person requiring complex treatment. Slightly less than one quarter of people in middle age had healthy periodontium, and 5% needed some hygiene instruction. 45% of group two presented with dental calculus, and 17.5% had at least one tooth with periodontal pocket from 3.5 to 5.5 mm; it means that main periodontal treatment need for people aged 35-44 was scaling (TN 2). The prevalence of periodontal pockets ≥6 mm was revealed in 10% of this group, they were classified for complex periodontal treatment. The study showed also that 17.2% of elderly subjects had no treatment needs, similar part of this group needed some hygiene instruction. For calculus these percentages were 34.5% and for periodontal pockets 3.5-5.5 mm, respectively, 24.2%. Nearly 7% of individuals aged 65-74 needed complex periodontal treatment (TN 3) because of presence of at least one tooth with deep periodontal pocket (*Tab. 3*).

Discussion

The periodontal condition of 18 year olds was evaluated in Poland in 1995. That epidemiological study revealed 18.5% subjects with healthy periodontium, 32.4% with gingival bleeding, 44.7% with dental calculus and over 4.4% with periodontal pockets [8]. Our data showed large number of young people with no treatment needs. This particular group consisted mostly of women which might have some influence on such good status, because women usually care more for their health.

There were some studies evaluating prevalence of periodontal problems among students of medical universities in Białystok and Warsaw [9,10]. Data from those studies differ: Popowski at al. [10] observed that 75% of sextants do not require any treatment needs, but Wawrzyn-Sobczak et al. [9] revealed only 48.86% healthy ones. In both group predominated treatment need was scaling (TN 2). Only 0.3% medical students had deep periodontal pockets and needed periodontal complex treatment. Popowski et al. [10] found that CPITN code 0 was revealed most often in upper middle sextant; the less healthy was lower middle one.

Stokowska et al. [11] evaluated the condition and treatment needs of adult individuals aged 35-44 living in the former district of Białystok in 1995. In that study 10.3% of sextants were healthy,

Table 3. **Percentage of peolpe according to CPITN code and category of treatment needs**

Group		CPITN				
		0	**1**	**2**	**3**	**4**
18	n (%)	149 (92.0%)	1 (0.60%)	7 (4.30%)	5 (3.10%)	0 (0%)
35-44	n (%)	9 (22.50%)	2 (5.0%)	18 (45.0%)	7 (17.50%)	4 (10.0%)
65-74	n (%)	5 (17.20%)	5 (17.20%)	10 (34.50%)	7 (24.20%)	2 (6.90%)
Group		**TN**				
		0	**1**	**2**	**3**	
18	n (%)	149 (92.0%)	1 (0.60%)	12 (7.40%)	0 (0%)	
35-44	n (%)	9 (22.50%)	2 (5.0%)	25 (62.50%)	4 (10.0%)	
65-74	n (%)	5 (17.20%)	5 (17.20%)	17 (58.70%)	2 (6.90%)	

comparing to 37.1% in ours. Periodontal disease (code 3 + 4) concerned, respectively, 34.4% and 27.5% of subjects. But our data show that severity of periodontal disease in Podlasie region increased during last years: in 1995 Stokowska et al. [11] found 1.1% of group with TN 3, eight years later it was 10%. Jańczuk [12] presented a comparison of data from epidemiological studies conducted in 1987 and 1995. He emphasized improvement of periodontal status in population of 35-44 year old persons. According to Konopka et al. [13] condition of periodontium of people living in Wrocław improved during first four years of last decade, they noticed higher score of CPITN codes 0 and 1, as well as lower percentages of code 4 than in previous study. Their observations confirmed that the dental plaque played important role in development of periodontal diseases, but the aggressive types of periodontitis had more complex reason. Brodeur et al. [14] examined 2110 Quebec adults aged 35-44 and revealed that 21.4% of Canadian population had at least one tooth with 6 mm or deeper pocket; similar study in Germany gave the result of 16.6% of subjects with this problem [15]. Besides in both studies prevalence of periodontal disease was higher than in our study, more than half people examined by Brodeur et al. had CPITN code 3.

It is widely assumed that symptoms of periodontal diseases escalate with age. Our study confirmed observation that *periodontitis* played an important role in the tooth loss in people after fourth decade of life. There were no excluded sextants in the youngest group, and more than half in the oldest one. Furthermore, one quarter of this group was endentouls, which was higher score than in similar study in France, but less than was found by Pasternak in region of Cracow [3,16]. Pasternak did not observed any sextant with CPITN code 0 and 1, but gingival pockets were stated in 86.7% of that population. Also people over 60 living in region of Gdańsk presented with worst periodontal condition than in our study [17].

Conclusions

Comparing to previous studies conducted in Poland 18 year old presented with healthy periodontium. The number of subjects with TN 3 revealed in population of people in middle age was higher than in similar studies. The role of periodontal diseases as a factor of tooth loss grows with age, based on large number of excluded sexstants noticed in the oldest group.

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Students′ knowledge of oral hygiene vs its use in practice

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Abstract

Purpose: The purpose of the work is to estimate the knowledge connected with the rules of the oral hygiene and its correlation with everyday habits among the students of Dental studies and Medical Studies at Medical School in Lublin and Polytechnics of Lublin.

Material and methods: A survey was conducted among 483 students: 58 2nd-year and 88 5th-year students of dentistry, 97 2nd-year and 51 5th-year students of medicine and 108 2nd-year and 81 5th-year students from The Polytechnics of Lublin.

Results: The study revealed that 50% students of dentistry, 32.43% students of medicine and 26.6% students of polytechnics brush their teeth after every meal; 94.23% students of dentistry, 89.91% students of medicine and 78.8% students of polytechnics know-how often teeth should be brushed. Students had better knowledge of how frequent they should change a toothbrush: 71.8% students of dentistry, 61.49% students of medicine and 54.4% students of polytechnics change their toothbrushes every 3 months, however, 84.61%, 62.16% and 49.42% students respectively have knowledge concerning the frequency of changing a toothbrush. The study also revealed that 13.46% students of dentistry, 10.14% students of medicine and 6.49% students of polytechnics visit dental clinic every 3 months, however, 4.49%, 13.51% and 14.05% students respectively go to see the dentist less than once a year. The reason for making a dental appointment was pain in 7.05% students of dentistry, 16.22% students of medicine and 22.22% students of polytechnics and a check-up in 64.74%, 62.84% and 51.85% students respectively.

Conclusions: Students' knowledge of oral hygiene does not always correlate with practice.

Key words: oral hygiene, students, questionnaire.

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Introduction

Hygiene (from Greek *hygienos*) is a science concerned with the investigations of environmental factors that affect human health. It studies how the human body responds to them. Obeying the rules of proper oral hygiene is of primary importance in the prevention of dental caries and periodontal diseases. Health education is also very important in dentistry. It is a basic issue of modern prevention programs and is included in all curricula of medical courses [1-3].

Dental health education is one of the preventive measures. Its aim is to raise awareness and motivate pro-health behaviors to maintain good oral health. The knowledge of prophylactic rules and their application in practice, necessary knowledge and thorough information adjusted to the patient's age are of high impact in accomplishing good oral health [3-7].

Purpose

The purpose of the work is to estimate the knowledge connected with the rules of the oral hygiene and its correlation with everyday habits among the students of Dental studies and Medical Studies at Medical School in Lublin and Polytechnics of Lublin.

Material and methods

A survey was conducted among 483 students: 58 2nd-year and 88 5th-year students of dentistry, 97 2nd-year and 51 5th-year students of medicine and 108 2nd-year and 81 5th-year

***Table 1*. How often do you visit dental office? (in %)**

Answer	Students of dentistry			Students of medicine			Students of polytechnics		
	2nd-year	5th-year	Total	2nd-year	5th-year	Total	2nd-year	5th-year	Total
Every 3 months	7.4	18.2	13.46	11.3	7.8	10.14	7.5	3.8	6.49
Every 6 months	63.2	59.1	60.9	49.5	47.1	48.65	41.1	34.6	38.38
Once a year	25.0	18.2	21.15	26.8	29.4	27.7	33.6	52.6	41.62
Less than once a year	4.4	4.5	4.49	12.4	15.7	13.51	17.8	9.0	14.05

***Table 2*. What influences the frequency of dental visits? (in %)**

Answer	Students of dentistry			Students of medicine			Students of polytechnics		
	2nd-year	5th-year	Total	2nd-year	5th-year	Total	2nd-year	5th-year	Total
Fear	7.5	17.9	14.74	9.5	1.9	8.11	13.3	15.1	14.09
Lack of financial means	3.0	6.4	5.77	6.3	1.9	4.73	12.5	16.1	14.09
Lack of time	28.4	17.9	22.44	25.3	32.1	25.68	24.2	17.2	21.13
No need	61.2	57.7	57.05	58.9	64.2	60.81	50.0	51.6	50.70

***Table 3*. What are the causes of making a dental appointment? (in %)**

Answer	Students of dentistry			Students of medicine			Students of polytechnics		
	2nd-year	5th-year	Total	2nd-year	5th-year	Total	2nd-year	5th-year	Total
Dental appointment	66.7	63.6	64.74	64.6	59.6	62.84	47.3	58.2	51.85
Dental caries	13.0	30.7	23.08	11.1	21.2	14.87	14.5	25.3	19.05
Pain	11.6	3.4	7.05	16.2	15.4	16.22	31.8	8.9	22.22
Rother causes	8.7	2.3	5.13	8.1	3.8	6.76	6.4	7.6	6.88

students from The Polytechnics of Lublin. The questions concerned the knowledge of proper pro-health behaviors in the area of oral hygiene and their use in practice.

The results were analyzed statistically by Chi^2 test of goodness of fit [8,9].

Results and discussion

The results are presented in *Tab. 1-8*.

Tab. 1 presents the results concerning the frequency of dental visits. They revealed that 60.9% students of dentistry (63.2% 2nd-year and 59.1% 5th-year students), 48.65% students of medicine (49.5% 2nd-year and 47.1% 5th-year students) and 38.38% students of polytechnics (41.1% 2nd-year and 34.6% 5th-year students) go to the dentist every 6 months. Chi^2 test found statistically significant differences among the students of dentistry, medicine and polytechnics concerning the answer of going to the dentist once or less than once a year: 21.15%, 27.7% and 41.62% students respectively go to the dentist once a year ($Chi^2=15.075$, $p=0.0005$); 4.49% students of dentistry, 13.51% students of medicine and 14.05% students of polytechnics go to the dentist less than once a year ($Chi^2=16.470$, $p=0.00026$).

Tab. 2 presents the causes of such frequency of dental visits. Most often mentioned cause was no need to see the dentist: 57.05% students of dentistry, 60.81%% students of medicine and 50.7% students of polytechnics. Another cause was lack of time mentioned by 22.4% students of dentistry, 25.68% students of medicine and 21.13% students of polytechnics. Fear of dental visit was mentioned by 14.74% students of dentistry, 8.11% students of medicine and 14.09% students of polytechnics. Additionally the students of polytechnics reported lack of financial means among the causes (14.09%).

Tab. 3 lists most common factors that motivate to make a dental appointment. Dental check-up was mentioned by 64.7% students of dentistry (66.7% 2nd-year and 63.6% 5th-year students), 62.84% students of medicine (64.6% 2nd-year and 59.6% 5th-year students) and 51.85% students of polytechnics (47.3% 2nd-year and 58.2% 5th-year students). Dental caries was the cause for 23.08% students of dentistry (13.0% 2nd-year and 30.7% 5th-year students), 14.87% students of medicine (11.1% 2nd-year and 21.2% 5th-year students) and 19.05% students of polytechnics (14.5% 2nd-year and 25.3% 5th-year students). Chi^2 test revealed statistically significant differences among the students of dentistry, medicine and polytechnics as far as pain being the cause of dental appointment was concerned: 7.05%, 16.22% and 22.22% respectively ($Chi^2=13.2501$, $p=0.0013$).

Other questions concerned the rules of oral hygiene. *Tab. 4* presents the answers to the question asking about the frequency of toothbrushing. Chi^2 test found statistically significant differences among the students of dentistry, medicine and polytechnics: 50%, 32.43% and 26.6% students respectively brush their teeth after every meal, 46.8%, 61.49% and 65.64% students respectively brush their teeth twice daily ($Chi^2=11.454$, $p=0.0032$); 1.92%, 4.73% and 5.13% students respectively brush their teeth once daily and 1.28%, 1.35% and 2.56% students respectively brush their teeth less than once a day.

Table 4. **How often do you brush your teeth? (in %)**

Answer	Students of dentistry			Students of medicine			Students of polytechnics		
	2nd-year	5th-year	Total	2nd-year	5th-year	Total	2nd-year	5th-year	Total
After every meal	44.1	54.5	50.0	29.2	39.2	32.43	22.0	32.6	26.67
Twice daily	50.0	44.3	46.8	63.5	56.9	61.49	66.1	65.5	65.64
Once daily	4.4	0.0	1.92	5.2	3.9	4.73	7.3	2.3	5.13
Less than once daily	1.5	1.1	1.28	2.1	0.0	1.35	4.6	0.0	2.56

Table 5. **How often teeth should be brushed? (in %)**

Answer	Students of dentistry			Students of medicine			Students of polytechnics		
	2nd-year	5th-year	Total	2nd-year	5th-year	Total	2nd-year	5th-year	Total
After every meal	97.1	91.9	94.23	90.5	90.2	89.19	75.0	83.8	78.8
Twice daily	2.9	8.1	5.77	8.4	9.8	10.14	20.2	16.3	18.48
Once daily	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.54
Less than once daily	0.0	0.0	0.0	1.1	0.0	0.68	3.8	0.0	2.17

Table 6. **How often do you change your toothbrush? (in %)**

Answer	Students of dentistry			Students of medicine			Students of polytechnics		
	2nd-year	5th-year	Total	2nd-year	5th-year	Total	2nd-year	5th-year	Total
Every month	10.9	12.6	10.26	12.5	9.8	11.49	20.6	16.0	18.68
Every 3 months	71.9	75.9	71.8	68.8	47.1	61.49	49.5	61.3	54.4
Every 6 months	12.5	9.2	10.26	12.5	31.4	18.92	18.7	17.3	18.13
Less than 6 months	4.7	2.3	3.21	6.3	11.8	8.12	11.2	5.3	8.79

Table 7. **How often a toothbrush should be changed? (in %)**

Answer	Students of dentistry			Students of medicine			Students of polytechnics		
	2nd-year	5th-year	Total	2nd-year	5th-year	Total	2nd-year	5th-year	Total
Every month	12.3	9.3	10.26	36.5	21.6	31.08	38.9	30.8	35.48
Every 3 months	86.2	88.4	84.61	58.3	68.6	62.16	44.4	56.4	49.42
Every 6 months	1.5	1.2	1.28	2.1	9.8	4.73	11.1	10.3	10.75
Less than 6 months	0.0	1.2	0.64	3.1	0.0	2.03	5.6	2.6	4.3

Tab. 5 presents the answers concerning the students' knowledge of adequate frequency of toothbrushing. The students of dentistry – 94.23% (97.1% 2nd-year and 91.9% 5th-year students), 89.19% students of medicine (90.5% 2nd-year and 90.2% 5th-year students) and 78.8% students of polytechnics (75.0% 2nd-year and 83.8% 5th-year students) knew that teeth should be brushed after every meal. Chi^2 test revealed statistically significant differences among the students of dentistry, medicine and polytechnics concerning the rule of toothbrushing twice daily: 5.77%, 10.14% and 18.48% students respectively said "yes" to that question (Chi^2 =12.210, p=0.0022).

Tab. 6 lists answers to the question asking about the frequency of changing one's toothbrush. The results found that 71.8% students of dentistry (71.9% 2nd-year and 75.95% 5th-year students), 61.49% students of medicine (68.8% 2nd-year and 47.1% 5th-year students) and 54.4% students of polytechnics (49.5% 2nd-year and 61.3% 5th-year students) change their toothbrush every three months. Chi^2 test found statistically significant differences concerning the answer of changing one's toothbrush every month among the students of dentistry, medicine and polytechnics: 10.26%, 11.49% and 18.68% students respectively (Chi^2=6.370, p=0.041).

Tab. 7 presents the answers concerning the frequency of changing one's toothbrush. Chi^2 test revealed statistically significant differences among the students of dentistry, medicine and polytechnics in the frequencies of changing one's toothbrush every month, every three months and every six months: 10.26%, 31.08% and 35.48% students respectively (Chi^2=20.532, p=0.00003) said that a toothbrush should be changed every month, 84.61%, 62.16% and 49.42% students respectively (Chi^2=17.248, p=0.0017) said it should be changed every three months and 1.28%, 4.73% and 10.75% students respectively (Chi^2=12.233, p=0.0022) said the toothbrush should be changed every six months.

Tab. 8 gathers the answers concerning the sources of information about the rules of oral hygiene: 48.76% students of dentistry, 36.27% students of medicine and 27.27% students of polytechnics obtained the information from the dentist, mass media as the source of information were mentioned by 15.92%, 21.57% and 33.6% students respectively, different brochures

Table 8. **Where do you obtain information on oral hygiene rulet from? (in %)**

Answer	Students of dentistry			Students of medicine			Students of polytechnics		
	2nd-year	5th-year	Total	2nd-year	5th-year	Total	2nd-year	5th-year	Total
From doctor	35.8	60.4	48.76	35.0	38.8	36.27	22.9	32.7	27.27
From mass media	16.8	15.1	15.92	21.9	20.9	21.57	35.0	31.9	33.6
From brochures	33.7	22.6	27.86	18.2	13.4	16.67	22.1	14.2	18.58
From family and acquaintances	13.7	1.9	7.46	24.8	26.9	24.49	20.0	21.2	20.55

were mentioned by 27.86%, 16.6% and 18.58% students respectively and 7.46%, 24.49% and 20.55% students respectively obtained such information from the family members or their acquaintances.

Conclusions

1. Students' knowledge of oral hygiene does not always correlate with practice.
2. Students of dentistry had the best knowledge of oral hygiene, then were the students of medicine and the students of polytechnics knew the least about oral hygiene.
3. The majority of students are aware that dental check-ups are important, however, a toothache continues to be the most common cause of making an appointment with the dentist.
4. There are different sources of information mentioned, however, the students of dentistry and medicine obtain it from the doctor mainly and the students of polytechnics from mass media.

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Evaluation of the results of periodontal treatment by means of digital subtraction of radiographic images

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Abstract

This article presents general principles and a sample case of application of digital subtraction of x-rays for objective evaluation of results of treatment in dentistry.

Purpose: Evaluation of the results of surgical periodontal treatment by means of digital subtraction of radiographic images taken before and 12 month after surgery.

Material and methods: For evaluation of the results of guided tissue regeneration treatment of deep bony defects digital periapical x-rays were taken before and 12 months after surgery. Pairs of images obtained during treatment were calibrated to equalize vertical x-ray beam angulation followed by calibration to radiological contrast and density. Next the comparison of images taken before and after treatment was performed by means of special computer software designed to subtract content of given images.

Results: Digital subtraction showed that the radiological density in regions where surgery was performed has decreased over a period of 12 months meaning that the mineral content which is responsible for absorbing x-ray photons has increased. Some local foci of subsurface hypomineralization were found on subtractions images. These foci couldn't be detected clinically because hypomineralization was taken place within bone.

Conclusions: Digital subtraction of x-rays taken before and after surgery treatment is detailed and objective method of evaluating results particularly when changes of surface and subsurface bone mineralization around teeth must be examined.

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Key words: digital subtraction, periodontal treatment, guided tissues regeneration.

Introduction

Radiological examination has been used in periodontology for many years. X-rays are used: to evaluate the level and condition of the alveolar ridge, to search for factors which may influence periodontal disease such as calculus, overhangs, etc., to prepare treatment plans and finally to evaluate the results of treatment. Panoramic images are generally employed to obtain informations about the general dental status while intraoral periapical images give detailed information regarding particular teeth and their supporting bone. Periapical views are therefore the images of choice for making a long-term evaluation of the results periodontal treatment particularly when surgical methods have been applied [1]. Radiological evaluation of the results of treatment is based on comparison of images of the same teeth taken "before" and "after" treatment. In the case of periodontal treatment "after" usually means several months or even years later. The time factor must be considered is such cases because changes in the degree of mineralization of the bone supporting teeth is directly connected with the changes in calcium and phosphate levels which may take a considerable time to appear. Ideally to make true comparisons between images, the following factors must be considered. Images should be obtained with the same vertical and horizontal angulation of the central x-ray beam, the same electrical settings – especially voltage (kV) and amperage (mA) and with the same x-ray machine, followed by standardised developing procedure involving similar conditions of temperature, time and quality of chemicals used [2]. Variations in any of the factors mentioned above will result in images of different contrast, density, tooth length may vary, location of interdental bone in relation to cemento-emanel junction may be incorrect. As a result it is impossible to make true comparisons. Comparison between "before" and "after" treatment images is based on the observer's perception which of course, may vary

between observers especially when one of them is the surgeon who carried out the treatment. Finally the results of such comparisons are difficult to present in a form of statistics which is used in international research publications. These limitations can be overcome by the use of another method of comparing images – a subtraction of digital images.

Digital subtraction overview

The rapid development of digital methods of visualisation in dentistry started in 1984 when Trophy, a French company, introduced the first digital x-ray sensor. Now digital x-rays are used in every day practice, in the form of a sensor or digital phosphor plate, which enables the practitioner to see images directly on a computer screen. Dentists who have some older "conventional" x-ray machines can now convert them to a digital form by means of flatbed scanners and are thus also able to see them directly on a computer screen. Some special programs can compare images and present the results in graphic or numerical form.

The digital subtraction method is based on the fact that every digital image is composed of very small particles called pixels and each of these pixels has only one colour. Pixels are so small that the human eye cannot see them separately but fuses them into one bigger part which may be seen as a mixture of different colours. Because radiographs consists of black, grey and white images the pixels which compose the image will range from black through grey to white. In fact, there are 256 different colours in the grey range of shades starting from 0, which is black, and ending on number 256, which is white [3]. It must be understood that a computer can "see" numbers instead of colours of pixels and it is only we, humans who need these graphic representations of numbers to "see something" on the screen. Once it is understood that these images are combinations of pixels with different numbers, some mathematical operations can be performed on them, the results can be presented in a numerical form and these can be used by computers, or in a graphic form which can to be seen by humans. When the computer program used for subtraction compare two images composed of identical pixels the resulting image will be grey. If on "after image" some new elements will be obtained a resulting image will show these new elements as whiter than the earlier ones. Also on some "after image" some elements may be less obvious or disappear. For example following periodontal bone loss the resulting image will show these elements as darker than earlier fragments. Data about gained or lost elements can be obtain in the form of numbers [4]. It must be remembered that these elements are pixels and that each pixel has its number. Despite the fact that the proper technique for taking a radiograph involves the use of holders for films or sensors, the correct vertical and horizontal angulation of the central x-ray beam and correct settings of the apparatus it is still almost impossible to obtain two absolutely identical images [5]. A great advantage of the computer program used is that it allows some "fine tuning" of the two images which are to be compared, before the procedure of digital subtraction is carried out. This is necessary because only pixels which are located in the identical position on both images can be compared and the operator of the program must give this information to the computer. The points usually chosen include the CEJ, apices of teeth, fragments of fillings etc. The more identical points on both images the better the quality of subtraction will be because the program can set the "after" image in the correct position in relation to the "before" image. This part of fine tuning of images can help to match images which were produced using slightly different vertical and horizontal angulation of the x-ray central beam. Once this has been done a carefull comparison of the density and contrast of the two images can be made. Author's experience is that most images have a slightly different density or contrast, or both. That is especially seen when conventional x-rays are converted to the digital mode by means of a flatbet scanner or when images are obtained by different digital sensors. That is why a second part of the fine tuning must be performed prior to subtraction. That step which may be called equalization of contrast and density allows the use a mathematical model of the average contrast and density of one image to be applied to another. After this second step has been taken the two images are ready for subtraction.

Figure 1. **Periapical x-ray image of lateral maxillary incisor before treatment**

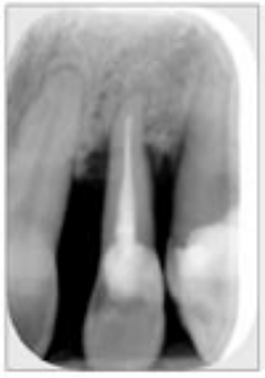
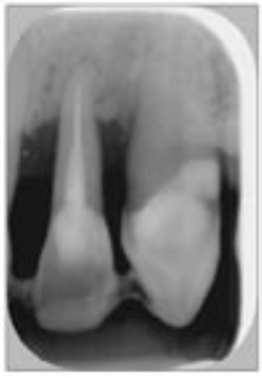


Figure 2. **Periapical x-ray image of lateral maxillary incisor 12 months after treatment matching**



The following section presents the digital evaluation of the results of surgical treatment of a periodontal defect in the region of lateral maxillary incisor by means of Emdogain.

The case

A patient aged 44 with a 9 mm periodontal pocket surrounding the left maxillary lateral incisor was referred to periodontal specialist for surgical treatment (*Fig. 1*). After completing hygienic procedures the teeth in region 21-24 were splinted by means on fiber/flow composite combination. This was followed by application of Emdogain into periodontal pocket round tooth 22 using the standard protocol. After soft tissue healing was complete the sutures were removed and the patient was advised about proper hygienic procedures and given a recall schedule. 12 months after surgery periapical digital radiograph of tooth 22 was taken to observe the results of the treatment (*Fig. 2*). It can be clearly seen that direct comparison of the two images is

Figure 3. Periapical x-ray image of lateral maxillary incisor before treatment with marked fixed points

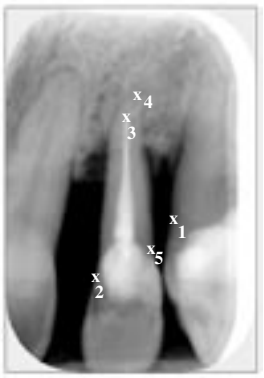


Figure 4. Periapical x-ray image of lateral maxillary incisor 12 months after treatment with marked fixed points

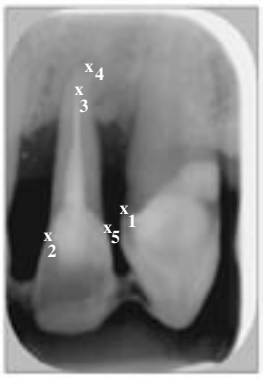


Figure 5. Image of lateral maxillary incisor 12 months after treatment modified according to fixed points

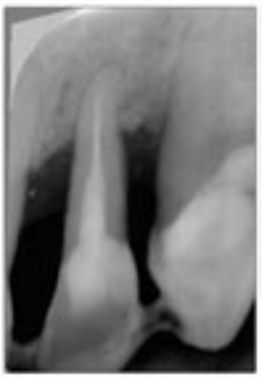


Figure 6. Image of lateral maxillary incisor 12 months after treatment modified according to fixed points and histogram matching

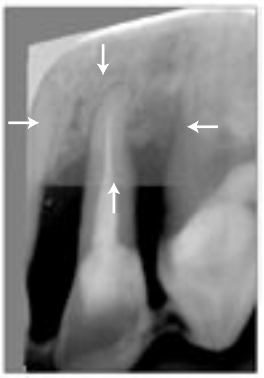


Figure 7. Effect of subtraction of "before" and "after" treatment x-ray images with colour filter

Figure 8. Periapical x-ray image of lateral maxillary incisor before treatment with marked regions of interest

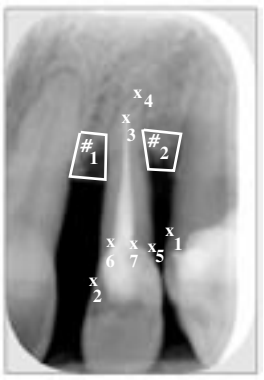
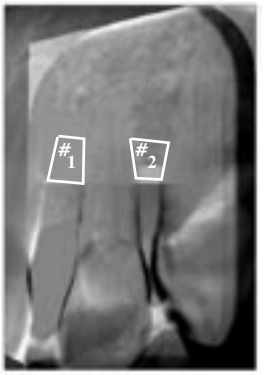


Figure 9. Image of subtraction of "before" and "after" treatment x-ray images with colour filter and marked regions of interest



very problematic. The two images were taken with slightly different vertical and horizontal angulations of the central x-ray beam. As a result the length of tooth 22 on the "before" and "after" treatment images is different, as well as the distance between the crowns of teeth 22 and 23. Analysis of the density and contrast of the images also shows differences. This can be especially well seen when the density of the root filling on both images is compared in relation to the density of the surrounding roots. Therefore any conclusions about the density of the bone and level in relation to length of tooth 22 are questionable. The application of digital subtraction program may overcome some of the problems mentioned above and provide some more accurate information about the result of treatment. To perform the analysis both images must be tuned by a computer program so the pixels located in the same position on both x-rays can be compared. First step in the procedure is alignment of the "after" image according to some fixed points present on both images. As mentioned in the previous part the more fixed points which can be used the better the degree of alignment that can be obtained. After marking fixed points (*Fig. 3*, *Fig. 4*) matching of images can be performed and the result can be seen on *Fig.* 5 By comparing *Fig. 4* and *5* changes in the length and shape of root of tooth 22 can be seen as well as differences in bone level. Because there are differences in density and contrast between the two images a second step, that of the equalization mentioned in previous part, must be performed prior to subtraction. After completing both these procedures the images are ready for the subtraction process. Superimposition of the two images and the effect of subtraction are presented in *Fig. 6*. Because of the different initial content of the "before" and "after" images some parts of final subtraction, i.e. the borders of the image, cannot be compared. The central part of *Fig. 6* where tooth 22 is located contains important information. The square part of the final image (marked with red arrows) represents the area were contrast/density equalization was performed. Since only that part of the final image contains true information about the effects of subtraction. For better visualization of changes a colour filter was used to show areas where higher (blue) and lower (yellow) mineralization are present. Unchanged areas are grey-coloured (*Fig. 7*). A graphic form of presenting

Table 1. **Grey level changes in regions 1 and 2**

Region	Type	Mean	Varians	Skew	Min	Max	Area	MaxCount	MaxCountAt
1	Gain	160.4336	104.8561	0.51599	136	192	2198	105	157
	Unch	139.0367	52.31566	0.79442	116	181	735	61	141
	Loss	116	3	0.3849	114	119	6	2	1
2	Gain	151.826	28.45347	0.328646	141	165	523	44	147
	Unch	132.1751	69.58103	0.096635	111	153	1491	70	127
	Loss	110.8997	29.24077	-0.28153	96	123	299	29	109

results can be obtained by the use of data which may be used for statistical analysis. To obtain proper data, particular areas of interest should be marked on either the "after" or the "before" image. An example of such regions marked on "before" image is presented in *Fig. 8*. and the final subtraction with the region of interest from where the data was collected is shown in *Fig. 9*. The data from regions 1 and 2 are presented in *Tab. 1*.

Results and concluding remarks

From the data presented it can be concluded that the radiological density in these regions has decreased over a period of 12 months meaning that the mineral content which is responsible for absorbing x-ray photons has increased. This is especially well seen in region 1 where both the gain/loss ratio, as well as the area of gain/area of loss ratio are high. In region 2 both hyper and hypomineralization can be observed. Although the gain/loss ratio is high, the area of gain/area of loss ratio is much lower. The observations about region 2, especially those concerning the presence of local foci of hypomineralization, are of great importance. These foci cannot be detected clinically because the process is within bone. Spreading of subsurface demineralization may reduce the positive result of this particular form of surgical treatment meaning that in this particular case actually increasing the depth of the periodontal pocket on the distal aspect of the lateral incisor. On the other hand the hypomineralization detected may be due to the bone remodelling phenomenon which can be often observed in regions where surgical treatment has been performed. Further x-ray observations are necessary to support this assumption fully.

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The relationship between mineral status of the organism and the number of teeth present and periodontal condition in postmenopausal patients

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Abstract

Purpose: The determination of the relationship between the mineral status of the organism and the number of teeth present and periodontal condition in women after menopause.

Material and methods: The study covered 65 postmenopausal women with partial loss of dentition, mean age was 66.2 years. The group was divided into 3 subgroups: healthy, with osteopenia and with osteoporosis. The division was made on the basis of the results of densitometric analysis (BMD) of femoral neck (F) and the lumbar spine (L2-L4), according to diagnostic criteria concerning the density of bone mass according to WHO. The number of teeth present was taken into consideration in the clinical examination. Periodontal condition was evaluated using CPITN index.

Results: The total number of own teeth strongly negatively correlated with the results of the lumbar spine densitometry. The correlation between mineral density of the lumbar spine and the femoral neck and the number of teeth in the maxilla was also strongly negative. However, the significant relationship between the number of teeth present in the mandible and the mineral density of examined bones was not observed. We did not state the increase in periodontal changes advancement together with the decrease in mineral status in the examined group of women.

Conclusions: There was not any influence observed of the decreased mineral status of the organism on the number of own teeth and the degree of periodontal disease advancement.

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Key words: mineral status of the organism, teeth, periodontium, menopause.

Introduction

Atrophy of the jaw bones is caused by numerous factors. However, so far the prevalence of either local or general factors in the etiopathogenesis of this process has not been determined. According to many authors, general loss of bone tissue in the course of metabolic bone disorders affects the masticatory organ. It can be reflected by early teeth loss, periodontitis intensification and advanced basal bone atrophy [1-4]. On the other hand, some authors claim that locally acting forces, together with systemic factors, modifying bone tissue resorption, are most vital in the pathogenesis of maxillary and mandibular bone atrophy [5-7]. Still others think that the decrease in mineral status has only indirect effect on bone atrophy, through acceleration of teeth loss and intensified course of periodontitis [8-12].

The aim of the study was the determination of the relationship between the mineral status of the organism and the number of own teeth and periodontal condition in postmenopausal patients.

Material and methods

The study was conducted in the group of 65 postmenopausal women with partial lack of dentition. The mean age of patients was 66.2 years.

The group was divided into 3 subgroups: healthy, osteopenic and osteoporotic. The division was made on the basis of the results of the densitometric examination (BMD) of the femoral neck (F) and the lumbar spine (L2-L4), following the criteria of diagnostic evaluation of bone mass density according to WHO [13].

Densitometric examination was performed using the method of Dual Energy X-ray Absorptiometry (DEXA) with

Table 1. **Mean number of teeth present in particular subgroups**

Subgroup	Number of teeth in maxilla	Number of teeth in mandible	Total number of teeth
Osteoporosis n=25	7.7±0.8	8.8±0.5	16.5±1.2
Osteopenia n=26	6.6±0.6	7.1±0.6	13.7±1
Healthy n=14	5.4±1.2	7.6±1	13±1.9

Table 2. **Correlation between bone mineral density of the lumbar spine (BMD L) and the femoral neck (BMD F) and the number of teeth in group with partial dentition**

Number of teeth	BMD L		BMD F	
	r	p	r	p
Total	-0.2537*	0.043*	-0.1931	0.126 n.s.
Maxilla	-0.2707*	0.031*	-0.2505*	0.046*
Mandible	-0.1697	0.18 n.s.	-0.0742	0.56 n.s.

r – correlation coefficient; * – statistically significant; n.s. – statistically insignificant

Table 3. **Periodontal condition in particular subgroups expressed in number (n), percentage (%), and the mean of sextants per person (m) according to CPITN index**

Group	Number of subjects	Number of sextants		Sextant excluded	Healthy periodontium	Gingival bleeding	Dental calculus	Pockets 3-5 mm	Pockets above 6 mm
				X	0	1	2	3	4
Healthy	14	84	n	37	7	12	20	8	-
			%	44	8.3	14.3	23.8	9.5	-
			m	2.6	0.5	0.9	1.4	0.6	-
Osteopenia	26	156	n	58	6	44	44	3	1
			%	37.1	3.8	28.2	28.2	1.9	0.6
			m	2.2	0.2	1.7	1.7	0.1	0.03
Osteoporosis	25	150	n	46	16	38	38	10	2
			%	30.6	10.7	25.3	25.3	6.7	1.3
			m	1.8	0.6	1.5	1.5	0.4	0.08
Total	65	390	n	141	29	94	102	21	3
			%	36.6	7.4	24.1	26.2	5.4	0.8

a bone densitometer Lunar DPX-L (USA). The results were expressed in g/cm^2.

The history data included the age, duration of menopause, lifestyle, nutritional habits. Patients with low body mass, secondary osteoporosis and other metabolic disorders as well as those treated with the hormonal replacement therapy were excluded from the study. The number of teeth present in the maxilla and in the mandible was taken into consideration during the clinical examination. Periodontal condition was assessed using Community Periodontological Index of Treatment Needs (CPITN).

The results were expressed as arithmetic means considering the standard deviation (SD). Pearson linear correlation coefficient was used to evaluate the interdependence between examined parameters. Correlation was considered to be significant at the values of $p<0.05$. The calculations were performed using Statistica 6 IBM.

Results

Tab. 1 presents the number of teeth present in patients in particular subgroups.

The highest number of teeth was noticed in patients with osteoporosis – 16.5±1.2 while the lowest – in the subgroup of healthy women – 13±1.9. The patients with osteopenia revealed the lowest number of teeth in the mandible – 7.1±0.6 whereas the healthy subgroup – 5.4±1.2 in the maxilla. The patients with osteoporosis had the highest number of teeth in the mandible and in the maxilla (8.8±0.5 and 7.7±0.8, respectively).

Tab. 2 shows the analysis of the relationship between the mineral condition, determined on the basis of mineral density examination of the femoral neck and the lumbar spine, and the number of teeth. The overall number of own teeth and the results of the densitometric examination of the lumbar spine were in the strong negative correlation. The strong negative correlation was also observed between the mineral density of the lumbar spine and the femoral neck and the number of teeth in the upper jaw. However, we did not find any significant relationship between the number of teeth present in the mandible and the mineral density of the examined bones.

Periodontal condition of examined subjects is presented in *Tab. 3*.

The condition of 390 sextants in general and in particular subgroups was assessed, out of which 141 sextants (36.6%) were excluded from the study. The mean number of excluded sextants was the highest in the subgroup of healthy women (2.6) while it was the lowest in the subgroup with osteoporosis (1.8). The subgroup with osteopenia revealed the lowest number of

sextants with healthy periodontium (CPI=0) (3.8%) while in the subgroup with osteoporosis it was the highest (10.7%) and in healthy comprised 8.3%. Gingival bleeding (CPI=1) and dental calculus (CPI=2) occurred least frequently in the subgroup of healthy persons (14.3% and 23.8%, respectively), the distribution was identical in the subgroup with osteopenia (CPI=1 – 28.2% and CPI=2 – 28.2%) and with osteoporosis (each CPI – 25.3%). The pockets of 3-5 mm (CPI=3) were present in 9.5% of healthy individuals, in 1.9% of patients with osteopenia and 6.7% of osteoporosis patients. The values were expressed per person as 0.6, 0.1, and 0,4 respectively. Advanced lesions in periodontium (CPI=4) did not occur in healthy controls, however in the subgroups with osteopenia and osteoporosis constituted 0.6% and 1.3%, respectively.

Discussion

The analysis of the relationship between the results of densitometric examinations and the number of teeth present showed strongly negative correlation between the results of the femoral neck and the lumbar spine density measurements and the number of teeth in the maxilla as well as between the result of the lumbar spine BMD and the total number of own teeth. We did not observe any statistically significant interdependence between the number of teeth in the mandible and the mineral density of the examined bones. Thus, it can be concluded that osteoporosis did not cause any accelerated loss of teeth. Moreover, the patients with lower BMD showed higher predisposition to keep the natural dentition, specifically in the mandible. It can be connected with more care of the oral health state. Klemetti et al. [6] did not connect the lowered mineral status with earlier or more intense loss of teeth. Mohammad et al. [14] did not present any correlation between spinal mineral density and the number of teeth although the indices concerning the intensity of periodontal disease were significantly higher in the group with low values of densitometric examination.

Rowe [15] analyzed the factors leading to the resorption of jaw bones, including all general factors affecting the decrease in bone mass and observed that tooth loss was the effect and not the cause of osteoporosis-derived resorption. The author claimed that the pattern of resorption was, among others, the result of innate predisposition to resorption at specific sites along the alveolar crest, which develops as the organism grows and later can favor teeth loss. Mattson et al. [16] stated that it was difficult to establish the direct connection between increased loss of teeth, bone atrophy or periodontal disease and osteoporosis as local symptoms pointed above occurred in some patients while others did not manifest any of them. Bando et al. [17] suggested that the satisfactory efficiency of mastication in patients with natural dentition and healthy periodontium might be a jaw bone protective factor against osteoporosis.

The relationship between the degree of periodontopathy intensity and osteoporosis has not been explicitly determined. Inagaki et al. [18] noticed the increase in periodontal disease intensity and advanced tooth loss in women with decreased mineral density of metacarpal bones. Von Wowern et al. [19] stated that advanced osteoporosis, which reduces to a great extent, the mineral content of the jaw bones, could be connected with lack of epithelial attachment of the gingiva. On the other hand, Wactawski-Wende [20,21] connected the decrease in mineral status only with chronic periodontal diseases. The author claimed that osteopenia could play a role in the pathogenesis of periodontopathy though the bacterial etiology of the disease had already been established. According to him, the same factors causing or modifying the course of both diseases (cigarette smoking, hyponutrition, age, corticosteroids applied or the immune system dysfunctions) constitute an important argument which confirmes the thesis. Baker [22] stressed that osteoporosis, like periodontal disorders, was connected with bone metabolism disturbances. A significant factor, that connects both ailments, is interleukin 6. Its level is increased as estrogen concentration decreases and in the course of periodontitis. If the diseases coexist, the increase in IL-6 level, connected with each of them, can enlarge the atrophy of the alveolar process. Periodontopathies are also intensified with age and generalized decrease in bone density can contribute to predisposition to the atrophy of the alveolar bone. Philstrom [23] claimed that osteoporosis is only a risk factor of periodontopathy occurrence in patients with poor oral hygiene and large amount of dental calculus.

Our results, based on CPITN, did not confirm the increase in periodontal disease advancement in people with lowered mineral status. The highest number of healthy sextants was stated in the subgroup with osteoporosis while the pockets of 3-5 mm in depth occurred more often in the healthy group. As far as most advanced changes in periodontium are concerned, they were not observed in healthy subjects and in the subgroups with osteopenia and osteoporosis, the percentage of sextants with the highest code was only 0.6% and 1.3%, respectively. The results were in accordance with other authors' reports. Klemetti et al. [48] also used CPITN index and stated its higher values (more advanced disease) in patients with better mineral status of the organism. However, the authors claimed that healthy individuals with deep periodontal pockets had less problems with maintaining their own dentition than patients with osteoporosis. Others did not find any direct relationship between the degree of periodontopathy intensity and osteoporosis advancement [24-26]. Norderyd et al. [27] observed the decrease in gingival pocket bleeding index in patients with the hormonal replacement therapy. However, neither the loss of the epithelial attachment of the gingiva nor bone resorption was lower in this group as compared to the patients without the hormonal replacement therapy. According to the authors, estrogen supplementation only enables the treatment of gingivae. Numerous differences of examination methods, both of mineral status and periodontal assessment, make it difficult to compare our results with that described in the literature.

Conclusions

The influence of decreased mineral status of the organism on the number of teeth present and on the degree of periodontal disease advancement was not observed in the examined group of women.

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Evaluation of periodontal status in young patients with insulin-dependent diabetes mellitus (type 1)

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Abstract

Purpose: **The aim of the study was to value periodontal status in young persons with well-controlled insulin-dependent diabetes mellitus (IDDM).**

Material and methods: **We examined 50 young people with IDDM (25 girls and 25 boys) and 50 healthy subjects (25 girls and 25 boys). Mean age of examined persons was about 14 years. We investigated gingival indexes: GI (Gingival Index) and PBI (Papillary Bleeding Index) and periodontal indexes: PI (Periodontal Index) and PDI (Periodontal Disease Index).**

The results were statistically analysed, and significant differences we observed for $p<0.05$.

Results: **The mean scores of Gingival Index and Papillary Bleeding Index were lower in healthy subjects but differences were not statistically significant. Only maximum scores of these indexes were significantly higher in diabetics.**

The mean and maximum values of Periodontal Index were significantly higher in patients with IDDM.

We did not notice differences between mean scores of PDI in both examined groups. Analysis of maximum values of Periodontal Disease Index reveals higher level in diabetic girls than in female controls.

Conclusions: **IDDM patients may be at risk of periodontal diseases. Well-controlling insulin-dependent diabetes mellitus may be important for periodontal tissues status and prophylaxis of periodontal diseases.**

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Key words: insulin-dependent diabetes mellitus (IDDM), periodontal status, children, adolescents, young adults.

Introduction

Diabetes mellitus is a pathological syndrome of varied etiology and diverse clinical course. World Health Organization has distinguished three types of this ailment: insulin-dependent (type 1), insulin-independent (type 2) and associated with other diseases and syndromes (type 3). Children and adolescents almost exclusively develop type 1 diabetes. Type 1 diabetic patients account for approximately 10% of all diabetics, of which 2% are under 18 years old. Epidemiological data of 1995 demonstrate that 8000 children were treated for diabetes in Poland [1]. According to the most recent surveys, 421 young patients were registered in the north-eastern region of Poland in the years 1988-1999. In other European countries, the incidence rate also shows a growing tendency [2].

Etiology of this ailment is not fully known. It is assumed that diabetes is most frequently caused by viral infection in genetically predisposed subjects and by autoimmunization directed against β cells of the pancreas that leads to their destruction (1). Viruses showing affinity to the pancreas are, e.g. viruses of hepatitis B, influenza, parainfluenza, cytomegaly and rubella. Other pathogens include many chemical substances as well as the mode of nutrition [1,3,4].

Diabetes can be diagnosed on the basis of such symptoms as excessive thirst and appetite, polyuria, body weight reduction, glucosuria and hyperglycaemia, confirmed by laboratory tests. In type 1 diabetes, when clinical symptoms appear, examination reveals damage to β islet cells with a resulting insulin deficiency [1,3].

High sensitivity to insulin usually makes insulin therapy indispensable. However, typical of the disease course in young patients is metabolic instability due to changeable demand for

Figure 1. **Mean and maximum values of Gingival Index (GI) and Papillary Bleeding Index (PBI) in diabetic and non-diabetic patients**

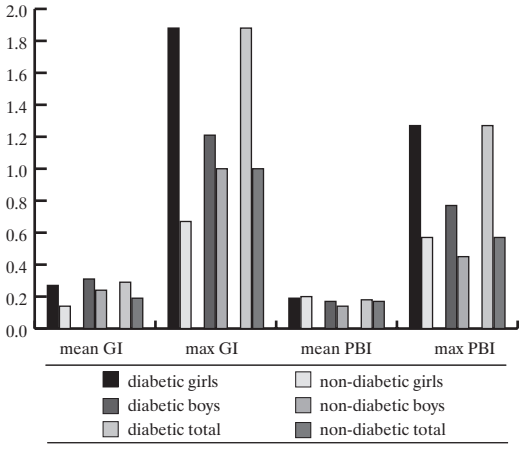
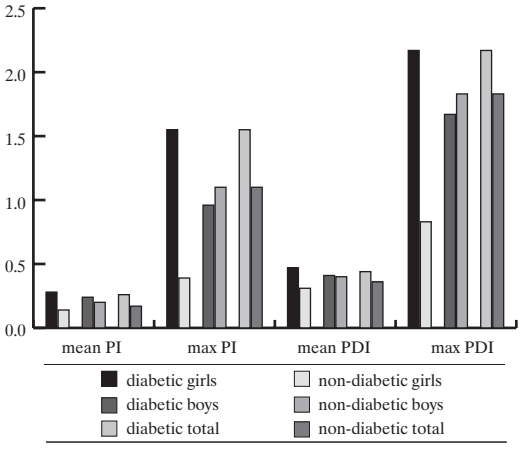


Figure 2. **Mean and maximum values of Periodontal Index (PI) and Periodontal Disease Index (PDI) in diabetics and non-diabetics**



insulin. Children and adolescents with well-controlled diabetes show normal physical and mental development [1].

However, some acute complications may develop in the course of diabetes, such as emergency conditions of deep metabolic disorders. The others are chronic, caused by changes in vessels, nerves and many organs, including the oral cavity [1].

Before insulin discovery, diabetes-related complications were common and markedly intensified. The periodontal status was characterized by inflammatory-atrophic changes, often with alveolar process necrosis and pyorrhoea. Nowadays, in well-controlled diabetes, oral lesions are milder and usually appear as gingivitis, which in case of metabolic instability can be severe [1,3-7].

The aim of the study was to assess periodontal status in children, adolescents and young adults with well-controlled type 1 diabetes.

Material and methods

The study involved 50 patients (25 female and 25 male subjects) with insulin-dependent diabetes treated in the Diabetic Outpatient Department Children's University Hospital in Białystok and 50 healthy individuals (25 female and 25 male subjects) treated at the Department of Pedodontics, Medical University of Białystok. The mean age of the study subjects was about 14 years. Examinations were performed in the dental surgery.

Periodontal status was determined based on:

- Gingival Index (GI) according to Löe and Silness (GI) [8],
- Papillary Bleeding Index (PBI) according to Saxers and Mühlemann [8],
- Periodontal Index (PI) according to Russel [8],
- Periodontal Disease Index (PDI) according to Ramfjord [8].

Test χ^2, Mann-Whitney test and t-Student test were used for statistical analysis of the results. Statistically significant differences were observed for $p<0.05$.

Results

Fig. 1 presents the mean and maximum scores of the gingival index. The mean GI score was lower in healthy subjects (0.19) than in diabetic patients (0.29). A similar correlation was noted in girls (diabetic 0.27, healthy 0.14) and boys (diabetic 0.31, healthy 0.24). The differences found were not statistically significant. The maximum GI level was significantly higher in diabetic patients (1.88) as compared to non-diabetic controls (0.67) ($p<0.05$). The correlation was similar for gender.

The mean Papillary Bleeding Index (PBI) did not differ significantly between the groups. The difference was only noted in the maximum PBI score, which was higher in diabetics (1.27) than healthy subjects (0.57), and significantly higher in diabetic girls compared to diabetic boys.

Damage to periodontal tissues was established based on PI and PDI. The mean and maximum scores are shown in *Fig. 2*. A significantly lower PI score was observed in healthy subjects (0.17) in comparison with diabetic population (0.26), and in the group of girls (healthy 0.14, diabetic 0.14). The maximum PI score was significantly higher in diabetic patients (1.55) as compared to healthy individuals (1.10).

The PDI score reflects the advancement stages of periodontal disease. The mean value did not differ significantly between diabetic patients (0.44) and non-diabetic controls (0.36). No statistically significant differences were noted in the mean PDI scores with reference to gender. The analysis of the maximum scores of PDI reveals higher level in diabetic girls (2.17) than in female controls (0.83) ($p<0.05$).

Discussion

Pathological changes in the periodontal tissue are late diabetic complications markedly determined by vascular lesions [9,10]. Capillaroscopy of the marginal gum vessels in diabetic patients revealed irregular distribution of loops, various length and thickness of blood vessels and degenerative changes in the pericapillary connective tissue. The basement membrane of periodontal capillaries was thickened, probably due to glycoproteid substance deposits. The above disorders hamper both oxygen diffusion and elimination of waste metabolites, thus disturbing physiologic equilibrium and increasing periodontium susceptibility to damage. A hyperglycaemia-induced increase in colagenase activity was also noted. Changes in collagen metabolism in the gum have an effect on the progression of periodontal destruction. Complications in periodontal tissues in the course of diabetes are also due to susceptibility to infection, delayed healing and immunity disorders, i.e. neutrophilic leukocyte dysfunction [11].

The GI is commonly used to define the status of the marginal periodontium. According to most researchers, diabetic patients have higher GI score compared to healthy population [12-20]. Only Andronikaki-Faldami et al. found similar GI values in young diabetics and non-diabetic controls [21]. Also in our study, the gingival index was only insignificantly higher in diabetic patients. However, the maximum GI score was significantly higher in diabetics, thus indicating that there were patients with more advanced gingivitis in the study group.

The Papillary Bleeding Index (PBI) reflects the condition of the marginal periodontium, especially in children and adolescents. However, it has not been yet assessed in young type 1 diabetics. We found a significant difference in the PBI maximum score, unfavourable for diabetic patients, which may suggest that a pathological process took place within the vessels of the marginal gum.

Literature data concerning the advancement of periodontal disease suggest more pronounced periodontal damage in diabetic children and adolescents as compared to healthy population [19,22]. In the studies carried out by Italian and Russian researchers [19,22], the mean PI and PDI scores in young diabetics were higher than in their healthy peers, which is in agreement with our findings, although in our study the differences were not statistically significant. Only for the maximum values, the PI and PDI levels were statistically significantly different, being higher in diabetic patients. However, no literature data have been available to compare with our results.

The present assessment of periodontal status as well as data reported by other authors allow the assumption that children, adolescents and young adults suffering from type 1 diabetes mellitus can be at risk of periodontal diseases [23]. Although Pinson et al. [15] claim that periodontal indices are not associated with the course of diabetes and periodontal diseases do not result from abnormal glucose metabolism, a vast majority of researchers emphasize a great role of proper diabetes monitoring [24-26]. It is believed that both hyper- and hypoglycaemia may induce the development of diabetic angiopathy and thus cause periodontal tissue dysfunction. Slight differences found in the present study in the periodontal status of diabetic patients, as compared to the control group, may be due to the fact that diabetes was systematically monitored and at the time of dental examination it was well-controlled.

It leaves no doubts, however, that in children and adolescents with type 1 diabetes, proper management of the primary disease should be accompanied by prevention, early detection and treatment of periodontal diseases.

Conclusions

1. Young type 1 diabetes mellitus patients may be at risk of periodontal diseases.
2. Properly-controlled diabetes may play an important role in periodontal tissues status and in the prophylaxis of periodontal diseases.

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The effect of glass ionomer cement Fuji IX on the hard tissues of teeth treated by sparing methods (ART and CMCR)

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Abstract

Purpose: The aim of the study was to assess the effect of glass ionomer fillings Fuji IX on the mineral content of the hard dental tissues of carious teeth treated by sparing methods.

Material and methods: The study material consisted of 4 deciduous teeth lost due to physiological resorption. The teeth had glass ionomer fillings Fuji IX inserted after treatment of caries by means of sparing methods (ART and CMCR). Chemical analysis of enamel and dentin was performed by means of energy dispersive spectroscopy (EDS) with X-ray analysis QUEST system at a distance of 20 um (point C) and 120 um (point D), respectively. The content of the following elements was evaluated in weight percent: oxygen (O), fluoride (F), sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), calcium (Ca), strontium (Sr). The Ca/P ratio was calculated. T-student test for pairs, with the level of significance $p<0.05$, was used for statistical analysis of the results.

Results: We found significantly higher levels of fluoride, aluminum and silicon and lower concentrations of calcium and phosphorus in the dentine adjacent to the filling (point C). However, no statistically significant differences were observed in the levels of the elements between these two sites of measurement.

Conclusions: Our results indicate that mineralization of the calcified dentine may involve elements released from glass ionomer cement Fuji IX.

Key words: glass ionomer Fuji IX, mineral analysis, dentin, enamel, BSE imaging.

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Introduction

Modern approach to the treatment of caries following the principles of minimum intervention recommends tooth sparing preparation [1]. Atraumatic restorative treatment (ART) and chemomechanical caries removal (CMCR) permit preservation of a substantial amount of the hard dental tissue. These techniques have been based on the studies by Fusayama [2] and Massler [3], who in the carious focus distinguished two calcified layers – the outer layer, which is infected, irreversibly denaturated and must be removed, and the inner layer remineralizable. Mineralization of the dentine left in the cavity is likely to occur due to the application of highly bioactive adhesive materials, such as glass ionomer cements.

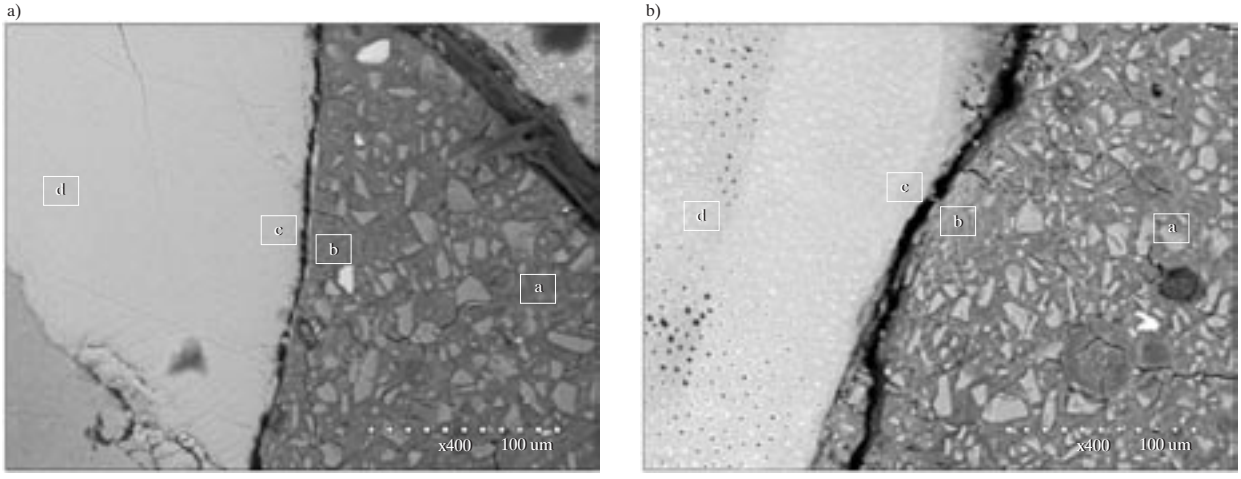
Since their introduction to clinical practice in 1976, glass ionomer cements have gained great popularity due to the release of fluoride ions whose anticariogenic effect is well known. In vitro, these restorative materials have been found to exert an effect on enamel remineralization and to attenuate enamel demineralization in the neighbourhood [7-12]. Some authors have demonstrated antibacterial properties of these cements against cariogenic microorganisms [13-15]. The studies by Forss [16], Wilson [17] and Ngo [18] have shown that not only fluoride but also other ions are released, including aluminum, sodium, silicon, calcium and strontium. Their effect on the hard dental tissues, especially on the partly calcified dentine is little known. Some study results seem to suggest that enamel apatites possess high capacity to exchange ions, and that remineralization does not always involve calcium and phosphorus supplementation [19].

The aim of the study was to assess the effect of glass ionomer cement Fuji IX on the mineral composition of the hard tissues of carious teeth treated by ART and CMCR methods.

Material and methods

The study material consisted of 4 deciduous anterior teeth, lost due to physiological resorption. The teeth had glass iono-

Figure 1. **Microscopic picture of a tooth with Fuji IX ond the borderline of: a) filling – enamel; b) filling dentin**



mer cement restorations Fuji IX, inserted after sparing cavity preparation (2 cavities for each sparing method). After the teeth had been filled, they were left in the mouth for 1-3 years.

Prior to examinations, the teeth were embedded in acrylic resin. Next, their surfaces were grinded on a rotary disc grinder with SIC grinding paper for 10 min and polished. The flat non-dust surfaces obtained in this manner showed distinct borderlines between the enamel, the dentine and the filling. An electron microscope Hitachi S-3000N (Japan) with an X-ray microbe analyzer Thermo Noran (USA) was used for analysis. The surfaces were photographed in BSE at 17kV voltage, at a magnification of 400x. Chemical analysis of enamel and dentin was performed by means of energy dispersive spectroscopy (EDS) with X-ray analysis Quest system on the sample surface area of 291.3 um^2 at a distance of 20 um (point C) and 120 um (point D), respectively – *Fig. 1*. The content of the following elements was assessed in weight percent: oxygen (O), fluoride (F), sodium (Na), magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), calcium (Ca), strontium (Sr). The Ca/P ratio was also calculated. T-student test for pairs, with the level of significance $p<0.05$, was used for statistical analysis of the results.

Results

The results of mineral analysis of enamel and dentine have been presented in *Tab. 1*. Dentine adjacent to the Fuji IX filling (point C) showed a significantly higher level of fluoride (F), aluminum (Al) and silicon (Si) compared to point D ($p<0.062$, $p<0.001$, $p<0.005$, respectively). The concentrations of calcium (Ca) and phosphorus (P) were significantly lower in the vicinity of the filling in comparison to the dentine at point D ($p<0.02$, $p<0.04$). The strontium content (Sr) was insignificantly higher at the filling. The content of oxygen (O), magnesium (Mg) and sodium (Na) expressed as a percentage of weight was practically the same in both sites of measurement. The elemental analysis of enamel revealed no statistically significant differences between the two sites. The Ca/P ratio approached 2.0 both for enamel and dentine and did not differ significantly between points C and D.

Discussion

Permeation of elements from restorative materials to dental tissues has been reported in several studies. Studies carried out in vitro by Hott et al. [20] and Extercate et al. [21] on bovine teeth revealed elevated fluoride levels in the dentine adjacent to glass ionomer fillings. Tveit et al. [22,23], who in vitro evaluated the absorption of fluoride released from amalgamates enriched with this element, found out that fluoride was better absorbed by dentine than enamel, the finding being consistent with our results. This, according to the authors, is associated with dentine structure, i.e. higher content of the organic part and water and lower crystallization rate. Mucai et al. [24], in vivo confirmed elevated fluoride levels in the dentine adjacent to Vitrabond fillings. Like us, they also found the highest concentration of fluoride directly under the filling, decreasing towards the pulpal surface, which indicates free penetration of this ion inside the dentine. However, conflicting evidence has been provided by Massara et al. [25], who found no presence of F ions in the dentine under the Fuji IX filling. Wessenberg and Hals [26], the Norwegians, assessing the effect of glass ionomer cement ASPA on the mineral composition of enamel and dentine of human teeth in vitro, determined the content of certain elements 1-3 months after the filling insertion. Our results are partly consistent with the data published by these authors. Like them, we found increased fluoride and aluminum levels in the tissues adjacent to glass ionomer cement fillings, the levels being statistically higher in dentine than in enamel. We also revealed similar concentrations of magnesium and sodium in the sites of measurement. Similarly to the Norwegians, we found no significant differences in the levels of Ca and P as well as in the Ca/P ratio in both sites within the enamel. However, some of our results were contradictory to theirs. Unlike them, we observed a rise in the concentration of silicon (Si) in the dentine adja-

Table 1. **The element content in weight percent in the study material**

Elements		FUJI IX			
		Dentin		Enamel	
		Point C	Point D	Point C	Point D
Mean	Oxygen (O)	40.88	40.43	44.37	41.68
SD		0.68	1.27	4.30	7.44
Mean	Fluoride (F)	0.87*	0.42*	0.62	0.50
SD		0.17	0.13	0.32	0.41
Level of significance		**0.006**			
Mean	Sodium (Na)	1.69	1.35	1.08	0.64
SD		2.28	1.64	0.43	0.15
Mean	Magnesium(Mg)	0.31	0.37	0.27	0.27
SD		0.27	0.25	0.12	0.14
Mean	Aluminium (AL.)	2.84*	1.08*	4.59	0.97
SD		0.44	0.45	5.73	0.30
Level of significance		**0.0001**			
Mean	Silikon (Si)	1.26*	0.82*	3.41	0.64
SD		0.49	0.39	4.43	0.23
Level of significance		**0.005**			
Mean	Strontium (Sr)	1.92	1.37	1.32	1.02
SD		1.42	0.77	0.56	0.50
Mean	Calcium (Ca)	34.11*	36.03*	31.75	36.01
SD		3.53	2.89	9.93	5.77
Level of significance		**0.025**			
Mean	Phosphorus (P)	17.44*	18.15*	15.60	18.27
SD		0.60	0.52	4.61	1.29
Level of significance		**0.040**			
Mean	Ca/P	1.95	1.98	2.03	1.97

cent to the filling. Knychalska-Karwan [27], Pawlicki [28] and Szczepańska [29], who were concerned with the assessment of Ca and P in the enamel of the deciduous teeth reported lower weight percent values of these elements than those obtained in our study. However, it should be remembered that the content of these elements in the hard dental tissues is changeable [30], and according to Pawlicki [28] in the deciduous teeth it undergoes variations with resorption progression. In our analysis, the levels of Ca and P in the dentin adjacent to the filling were significantly lower than those 120 um distant. Wesenberg and Hals obtained contradictory evidence for these elements [26]. However, they examined permanent and not deciduous teeth and in their study ASPA fillings were placed in experimentally formed cavities in the intact dental tissue. Massara et al. [25], who assessed ART, found elevated levels of Ca in the dentine close to Fuji IX, but their study methods differed from ours. The Ca/P ratio obtained in our study is almost identical with those presented by other authors assessing dentine composition after chemomechanical treatment of caries [31,32]. In the current study, the cavities were prepared using manual methods (ART and CMCR). The lower concentrations of calcium and phosphorus in the dentine directly under the filling as compared to the distant sites may suggest the presence of partly demineralized dentine on the cavity floor. This suggestion has also been made by Angker et al. [33].

The significant increase in weight percent values of fluorine, aluminum and silicon in the dentine adjacent to Fuji IX cement may indicate passing of these elements from the filling to the tissue. According to the most recent reports [18,34,35], certain elements such as aluminum, fluorine, strontium and silicon are likely to replace calcium and perhaps phosphorus in apatites. In conclussion the results of the current study seem to confirm the assumption that dentine remineralization may involve elements permeating from the glass ionomer cement Fuji IX into the tissue.

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Hygienic habits and the dental condition in 12-year-old children

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Abstract

Purpose: **The aim of the study was the description of the dental condition of hygienic routines in 12-year-old children in urban and rural areas of Lublin voivodship.**

Material and methods: **The study comprised 274 children at the age of 12 (152 girls and 122 boys). 95 girls and 92 boys came from the urban area; 57 girls and 30 boys came from the rural area.**

Results: **On the basis of clinical examination it was concluded that 11.96% of boys and 18.95% of girls from the urban area and 6.67% boys and 8.77% of girls from the rural area brush their teeth after every meal; 60.87% of boys and 68.42% of girls from the urban area and 43.33% of boys and 50.88% of girls from the rural area brush their teeth twice; 22.83% of boys and 11.58% of girls from the urban area and 26.67% of boys and 28.07% of girls from the rural area brush their teeth once daily.**

DMF count was for boys from the urban area – 4.12/girls – 3.92 and for boys from the rural area 4.50/girls – 4.29. The treatment indicator was for boys from the urban area – 0.56/girls – 0.47 and for boys from the rural area 0.35/girls – 0.67.

Conclusions: **On the basis of the research conducted in the study, it was concluded that tooth brushing is more frequent with urban area children than in children from rural area. This leads to a conclusion that the action for improvement of the health awareness is a dire need among 12-year-olds both from urban and from rural areas.**

Key words: 12-year-old children, dental condition, questionnaire.

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Introduction

Dental education is one of the prophylactic tasks aiming at increasing health awareness and motivation of pro-health actions in preserving of the health of the mastication organ. The knowledge pertaining to prophylaxis rules and the detailed information offered to different age groups of patients plays a crucial role in acquiring proper dental condition of the oral cavity [1-5].

Purpose

The aim of the study was the description of the dental condition of hygienic routines in 12-year-old children in urban and rural areas of Lublin voivodship.

Material and methods

The study comprised 274 children at the age of 12 (152 girls and 122 boys). 95 girls and 92 boys came from the urban area; 57 and 30 boys came from the rural area.

Clinical and questionnaire study were conducted. Clinical examination evaluated the state of dental condition while the questionnaire evaluated hygienic routines. The results obtained in the study were compared in subgroups by the application of Chi^2 independence test of Pearson.

Results

The results of the study are presented in *Tab. 1-6*.

In *Tab. 1* the data concerning the frequency of teeth brushing are collected. The application of Chi^2 independence test of Pearson (Chi^2 of Pearson's 33.3616, df=9, p=0.000116) allows describing the relevant differences in the teeth brushing frequency. The smallest frequency of tooth brushing is observed

Table 1. **The frequency of toothbrushing express in %**

	Boys – urban	Girls – urban	Boys – rural	Girls – rural
After etery meal	11.96	18.95	6.67	8.77
Twice a day	60.87	68.42	43.33	50.88
Once a day	22.83	11.58	26.67	28.07
Less frequent	4.35	1.05	23.33	12.28

Table 2. **The frequency of toothbrush exchanging in %**

	Boys – urban	Girls – urban	Boys – rural	Girls – rural
Every 3 months	51.65	69.47	43.33	57.89
Every 6 months	24.18	13.68	23.33	17.54
After complete usage	24.18	16.84	33.33	24.56

in boys from the rural areas – 23.33% of boys brush their teeth no more than once, the highest frequency pertaining to toothbrushing was observed in girls from the urban area – 18.95% of girls brush their teeth after every meal and 68.42% of girls do it twice a day.

In *Tab. 2* the data reflect the frequency of exchanging the old toothbrush into a new one. The data signify the fact that the toothbrush is regularly exchanged every 3 months by 51.65% of boys and 69.47% of girls from the urban areas and 43.33% and 57.89% of girls from the rural areas. Basing on Chi^2 independence test of Pearson (Chi^2 of Pearson's 10.0344, df=6, p=0.123225) no significant differences were reported as for the frequency of exchanging toothbrushes among children from rural and urban areas.

Tab. 3, presents data concerning the duration of brushing. The application of Chi^2 independence test of Pearson (Chi^2 of Pearson's 45.7221, df=12, p=0.000008) allowed reporting significant differences in the duration of brushing. 3-minute brushing is common with 33.70% of boys and 46.32% of girls from the urban area and in 23.33% of boys and 22.81% of girls from the rural area. 1-minute brushing was observed in 8.70% of boys and 2.11% of girls from the urban area and in 23.33% of boys and 33.33% of girls from the rural area. Brushing duration is ignored by 27.17% of boys and 22.11% of girls from the urban area and in 20.00% of boys and 8.77% of girls from the rural area.

Tab. 4 presents the data concerned with the type of the toothbrush used for brushing. The application of Chi^2 independence test of Pearson (Chi^2 of Pearson's 55.2200, df=15, p=0.000002) allowed reporting significant difference as for the application of different toothbrushes. Traditional toothbrush was common with 75.0% of boys and 74.74% of girls from the urban area and with 96.67% of boys and 89.48% of girls from the rural areas, while the electric toothbrush was more common with urban area among children – 25.0% of boys and 23.16% of girls than in rural area – 3.33% of boys and 10.53% of girls.

Tab. 5 presents the data of application of additional means of dental hygiene of the oral cavity. It was stated that additional means of dental hygiene of the oral cavity are sometimes applied by 89.13% of boys and 95.79% of girls in the urban area and by 100% of boys and 92.98% of girls in the rural area. None of the

Table 3. **Toothbrushing duration in %**

	Boys – urban	Girls – urban	Boys – rural	Girls – rural
3 min	33.70	46.32	23.33	22.81
2 min	30.43	28.42	33.33	35.09
3 or 2 min	0.00	1.05	0.0	0.00
1 min	8.70	2.11	23.33	33.33
Don't know	27.17	22.11	20.0	8.77

Table 4. **The use of traditional and electric toothbrush in %**

	Boys – urban	Girls – urban	Boys – rural	Girls – rural
Traditional	75.0	74.74	96.67	89.48
Electric	25.00	23.16	3.33	10.53
Traditional and electric	0.00	1.05	0.00	0.00

Table 5. **The application of additional means of dental hygienic**

	Boys – urban	Girls – urban	Boys – rural	Girls – rural
Dental floss, liquid	7.32%	4.40%	0.00%	1.89%
Liquid	31.71%	36.26%	66.67%	69.81%
Dental floss	24.39%	38.46%	3.33%	11.32%
Toothpick	28.05%	9.89%	20.00%	15.09%
Dental floss, toothpick	3.66%	3.30%	0.00%	0.00%
Irrigator	1.22%	0.00%	0.00%	0.00%
Liquid, toothpick	3.66%	3.30%	10.00%	1.89%
Dental floks, liquid, toothpick	0.00%	4.40%	0.00%	0.00%

questioned children reported an everyday use of the additional means of dental hygiene. The application of Chi^2 independence test of Pearson (Chi^2 of Pearson's 59.4853, df=21, p=0.000015) allowed reporting significant differences in the percentage of children applying the additional means of dental hygiene. The most frequent means was the oral cavity cleansing and refreshing liquid applied by 31.71% of boys and 36.26% of girls from the urban areas and 66.67% of boys and 69.81% of girls from the rural areas, the least frequent means of dental hygiene was the irrigator applied only by 1.22% of boys from the urban areas.

Tab. 6 presents the data concerned with DMF count and its components D, M, F and the treatment indicator. The analysis of these data showed that DMF count was close in all research subjects and was 4.12 in boys and 3.92 in girls from the urban areas and 4.50 in boys and 4.29 in girls from the rural areas. However, there were the differences in the treatment indicator, which was for boys from the urban area – 0.56/girls – 0.47 and for boys from the rural area 0.35/girls – 0.67.

The frequency of dental caries among the studied group of youth was 73.91% in boys and 80.0% in girls from the urban area and 100% in boys and 78.95% in girls from the rural area.

Totally there was 1.09% of children at the age of 12 with the extracted permanent tooth due to caries; in the urban area the percentage for such cases was 1.09% in boys and 2.11% in girls while in the rural area the percentage was 2.11% in boys and 0% in girls.

Table 6. **DMF count and treatment indicator**

	Boys – urban	Girls – urban	Boys – rural	Girls – rural
D	1.73	2.01	3.0	1.48
M	0.01	0.02	0.0	0.0
F	2.55	1.9	1.5	2.80
DMF	4.12	3.92	4.50	4.29
F/D+F	0.56	0.47	0.35	0.67
The frequency of dental	73.91%	80.0%	100.0%	78.95%

Discussion

Mielnik-Błaszczak et al. studying 12-year-old children from the rural areas in Lublin voivodship in 1998 concluded that 2.9% brushed their teeth after every meal, 52.9% brushed their teeth twice a day, and 29.4% exchanged their old toothbrush. In our studies we stated that the greater percentage of children from the rural area brush their teeth after every meal, which is from 6.67% to 8.77%. There is also a greater number of children exchanging their old toothbrush every three months – from 43.33% to 57.89% [8].

We also found out in our studies that there exist a greater percentage of children applying additional means of dental hygiene as compared with the results of Mielnik-Błaszczak et al. However, when we compare the frequency of toothbrushing by 12-year-old children from Lublin and Łódź area [9] we find out that in Łódź toothbrushing is common with 57% of children who do it twice a day and with 37% of children who do it once a day and 6% who do it rarely. In Lublin the data were as follows: 64.71%, 17.11% and 2.67%. When one compares the intensity of dental caries, however, it was found out that in boys in Łódź the DMF count was 3.38 and in girls – 2.63 as compared to Lublin 4.12 and 3.92.

The study of the children from urban and rural area from Mazovian voivodship, similarly to our findings, showed the absence of greater differences between children from rural and urban areas as for DMF count and also between the boys and girls [10]. Similar findings were achieved by Bachanek et al. who studied 12-year-olds children from the former Chełm voivodship [11]. The mean value of DMF count in our study – from 3.92 to 4.50 is within the norm for "mild intensification" of dental caries and is close to the count of mean DMF count for 12-year-old children in Poland [12]. Similarly as in the general studies for Poland, higher values of DMF in rural area children were stated as compared to urban area, there was a smaller percentage of 12-year-old children with an extracted tooth due to dental caries [12].

Conclusions

1. Tooth brushing is more common in urban areas children than in rural areas children.
2. Brushing is more common among girls than boys.
3. The highest treatment indicator was reported in girls from the rural areas and the lowest in boys from rural areas.
4. The proper action aimed at intensification of health awareness in 12-year-old children is needed in both rural and urban areas.

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Tobacco smoking problem in a group of 18-year-old high school students in the city of Gdańsk – finding causes and preventive methods

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Abstract

Purpose: The aim of the study was to evaluate smoking prevalence among 18-year-old secondary school students as well as their awareness of systemic health threats of smoking. Our goal was also to discuss the youth smoking risk factors and effective ways both to prevent and fight smoking problem.

Material and methods: 1516 18-year-old students (808 men, 708 women) from randomly selected 12 high schools were studied. The adolescents fulfilled the anonymus questionaire.

Results: 34.1% (517) of all participants smoke every day or occasionally, with the highest percentage of smokers in vocational schools (49.6%); women are the most frequent smokers (52.8%). The lower prevalence of smoking was observed in high schools (21.2% of men, 20% of women). In technical high schools 36.1% of men and 11.1% of women were smokers. The habitual smokers were found in all schools; the highest percentage was observed in vocational schools (32.75%-33.13%). The percentage was particularly high among women (33.13%). 92.09% of studied women and 89.95% of men were aware of smoking systemic health threats (93.84% of high school students, 88.25% of vocational school students).

Conclusions: It is alarming that the percentage of smokers among 18-year-old students is high, in particular among women and vocational schools students. The results indicate that smoking is a serious problem in this population. It is vital to create the preventing and educating programmes addressed especially to adolescents. There is a need of future studies aimed to evaluate smoking risk factors and create effective methods of prevention as well as smoking cessation help resources.

Key words: adolescents, cigarette smoking, prevalence, psychosocial risk factors, prevention.

Introduction

Epidemiological studies of Polish population in the recent years show a high percentage of smokers among adults, teenagers and juveniles [1]. Wide range of systemic health threats tied to smoking habit, particularly cancers and cardiovascular diseases [2,3], proves a need to conduct intensification study of this phenomenon among Polish youth and to undertake educative and prophylactic measures.

Aim

The aim of the study was to evaluate smoking prevalence and frequency among 18-year-old secondary school students of different profiles and to find ways to fight the smoking problem.

Material and methods

Study was conducted in schools randomly selected by the Gdańsk City Office (7 high schools, 4 vocational schools and 1 technical high school). 1516 18-year-old students of both genders, 808 men and 708 women were examined. Youth had filled anonymous questionnaire about number of smoked cigarettes, smoking habit duration and awareness systemic health threats of smoking.

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Table 1. **Tobacco smoking intensification among students according to school profile and gender**

School Profile	Men		Women		Total number of students in school	Total number of smokers (%)
	Number of studied students	Number of smokers (%)	Number of studied students	Number of smokers (%)		
High school	344	73 (21.22%)	370	74 (20%)	714	147 (20.59%)
Vocational school	284	132 (46.48%)	329	172 (52.28%)	613	304 (49.59%)
Technical high school	180	65 (36.11%)	9	1 (11.11%)	189	66 (34.92%)
Total	808	270 (33.42%)	708	247 (34.89%)	1516	517 (34.10%)

Table 2. **Number of cigarettes smoked per day according to school profile**

School profile	Number of men studied	Number of men smoking		Number of women studied	Number of women smoking	
		Up to 10 cigarettes a day (%)	Over 10 cigarettes a day (%)		Up to 10 cigarettes a day (%)	Over 10 cigarettes a day (%)
High school	344	29 (8.43%)	44 (12.79%)	370	42 (11.35%)	32 (8.65%)
Vocational school	284	39 (13.73%)	93 (32.75%)	329	63 (19.15%)	109 (33.13%)
Technical high school	180	16 (8.89%)	49 (27.22%)	9	0 (0%)	1 (11.11%)
Total	808	84 (10.40%)	186 (23.02%)	708	105 (14.83%)	142 (20.06%)

Results

Results are presented in *Tab. 1-3*. Data in *Tab. 1* show, that among 1516 studied students 517 (34.1%) smoke every day or occasionally. The highest number of smokers was observed in vocational schools (49.6%), where the highest percentage of smokers was among women (52.8%). In high schools percentage of smokers in both genders was lower, similar – 21.2% in men and 20% in women. In technical high school men smoked more (36.1%) than women (11.1%).

In every school students who smoke over 10 cigarettes a day were found, they can be classified as habitual smokers. Mostly they were vocational schools students (32.75%-33.13%), women in particular (33.13%). (*Tab. 2*)

Positive answer to the question about being aware of systemic health threats of smoking was given by 726 men (89.85%) and 652 women (92.09%). The highest percentage of students knowing the systemic health threats were high school students (93.84%), the lowest in vocational schools (88.25%). (*Tab. 3*)

Discussion

Results of our study show that smoking among 18-years-old school youth, especially in vocational school, constitutes a significant problem calling for decisive prophylactic/preventive action. It seems to be even more important due to the fact that majority of the students studied were aware of negative influence of smoking on general health condition; however, this fact did not result in quitting smoking.

What are the factors that favour smoking?

Many research on correlates of cigarette smoking among children, adolescents and adults seem to emphasise multifactorial model containing social influence, environmental, psychological and behavioural factors [4,5]. One of the most important factors coexisting with smoking is positive attitude towards smoking as well as beliefs and opinions about positive consequences of nicotine intake. These opinions are shared by adolescents and, unfortunately, also by children [5-10]. Many of the adolescent smokers (68%) think that smoking helps to calm down when a person is upset, embarrassed or sad [6]. Research also proved that these opinions are even shared by non-smoking persons, with children among them, who consider smoking the way to improve mood. British study of 3000 children (aged 11-15) who never smoked revealed that about 60% of them believed smoking could be a helpful way to relax [6]. These results seem to indicate that positive attitude toward cigarette smoking doesn't have to be the result of personal experience but sometimes rises from suggestions and other people's opinions as well as from the observation of social behaviour. Teenagers reported that among several examined behaviours,

Table 3. **Level of students' awareness of unfavourable influence of smoking**

School Profile	Men		Women		Total number of studied students	Total number of aware students (%)
	Number of studied students	Number of aware students (%)	Number of studied students	Number of aware students (%)		
High school	344	321 (93.31%)	370	349 (94.32%)	714	670 (93.84%)
Vocational school	284	245 (86.27%)	329	296 (89.97%)	613	541 (88.25%)
Technical high school	180	160 (88.89%)	9	7 (77.78%)	189	167 (88.36%)
Total	808	726 (89.85%)	708	652 (92.09%)	1516	1378 (90.90%)

nicotine intake is the most frequent phenomenon observed in their environment [11]. Thus, the common presence of smoking behaviour can influence their decision to smoke.

Studies concentrated on environmental factors coexisting with smoking among adolescents emphasised the impact of peers' attitudes and behaviours [4,5]. The risk of initiation and smoking maintenance is significantly associated with smoking among adolescents' friends and siblings and their attitudes toward smoking.

Initiation and prevalence of smoking can also be influenced by family structure, parental attitudes toward smoking, parental smoking (especially mothers), the quality of parent-child relationship and parental attachment [4,7,9,10,12,13]. It's worth to emphasise that the poorer the relationship with parents, the stronger the adolescent's tendency to become peer group member and to be 'one of them'.

Research on reasons of initiation of cigarette smoking indicate that stress is one the most important correlates [4,9]. This factor seems to be especially important in political and economical transformation period in Poland. One study aimed to "evaluate" the reasons of smoking revealed that "dealing with stress" was the most frequently reported factor leading to smoking [9]. The authors emphasised the fact that reasons of smoking associated with stress and frustration, resulting from "missed" possibilities and lack of prospects, prevailing now among the youth, hadn't been mentioned by Polish adolescents in the 60s-80s. The researchers contribute the present tendency to the socio-political situation in our country.

Many of the researchers indicate the coincidence of smoking and other risk behaviours, e.g. alcohol or drug use [14-16]. According to these studies the problem of smoking among the adolescents becomes even more important, as it is defined as alcohol or drug addiction risk factor and proves the necessity of actions – not only preventive ones, but also those encouraging adolescents to quit smoking.

There has been noticed the co-morbidity of smoking or nicotine dependence and mental disorders [6,17]. This problem seems to be serious taking into consideration the results of Finish psychiatric inpatient adolescents study which revealed three times higher risk of self-mutilation and four-fold risk for suicidal thoughts and attempts among smokers than non-smokers [18]. Depression is one of the disorders that raise the risk of smoking initiation, correlated with number of smoked cigarettes and difficulties with cessation. Depressed people are also less likely to remain abstinent due to the fact that when a person is addicted to nicotine, worsened mood can be the withdrawal symptom [17]. Depression is also the predictor of future smoking. The above associations are observed in children, adolescents and adults [4,6,15,17,19-21]. Research results support the conclusion that perhaps we deal with such a problem in Poland. Study aimed to estimate prevalence of depression among Polish adolescents revealed depressive disorders in 50% students from comprehensive schools and 65% students from vocational schools. From these young people up to 25% required antidepressive treatment [22]. Moreover, alarming are the results of studies on mental health problems among adolescents between 13-17 years of age [23,24] who reported high level of sadness, gloom, loneliness, low self-esteem and suicidal thoughts. High prevalence of depression among 16-17 aged students revealed in research by Jaklewicz [22] and the high prevalence of smoking among our study population (18-years-old), especially in vocational schools, suggest the possibility of coexistence of the phenomenon in Polish adolescents, which have been reported by some authors [25]. The additional problem is that cigarette smoking can have the bearing on depressive mood increase [26]. Perhaps, we have to face the 'vicious circle' mechanism.

One of the most important psychological factors that have been consistently reported as associated with smoking is self-efficacy – the basic term phenomenon of Bandura's theory [27]. According to Bandura, self-efficacy refers to one's beliefs concerning his or her ability to cope with problems and anticipation of success in solving them. Research on association between self-efficacy and health behaviours with smoking among them, revealed that among children, adolescents and adults low self-efficacy leads to smoking initiation and maintenance as well as cessation failures [28,5]. World-wide research findings are consistent in these results. One of the most important aspects of self-efficacy is its influence on social functioning, because the level of self-efficacy determines the efficacy in resisting negative social pressure. In practice low self-efficacy means that a young person has no ability to resist his or her friends' negative influence and because of that the risk of the negative

behaviour increases. This 'refusal self-efficacy' is one of the protective factors against smoking initiation, continuing smoking and maintaining the abstinence [7,10]. Findings of research of Danish adolescents indicate that youth smoking initiation can be based on three-dimensional 'attitude-social influence-self-efficacy model' [5]. The authors of the study emphasised the role of increasing self-efficacy in smoking prevention process in adolescents.

Rotter's 'locus of control' (internal vs external) is another psychological factor that is believed to be related to health behaviours [29]. The person with more internal locus of control believes that he or she is responsible for his or her life, behaviour and action and has an ability to control the life. Such a person is more disposed to positive health – related behaviours and better compliance with medical treatment.

Cigarette smoking among adolescents as well as adults seems to be one of the main ways of dealing with stress [12]. Furthermore, smoking is used because of the lack of abilities to cope with stress with positive means. This problem should be of particular importance due to the fact that 'dealing with stress' is the most frequent smoking motivational factor reported by Polish adolescents [9] who have no knowledge about stress coping methods [30]. The problem seems to be even of greater importance that, as studies show, young people facing problems try to solve them on their own or ask for help and support their peers, friends and only later they spot adults, most often their parents, which is a positive sign. Taking the above into consideration, it seems necessary to develop and strengthen, in child's earliest years, the above mentioned personality traits and abilities helping in successful coping with problems, including frustration and stress, and as regards adults – realise the impact of parent – child relationship on their convictions, behaviour, actions and life choices.

How to prevent smoking?

The review of the literature on smoking in adolescents provides the clear evidence that there is a need of educational programs addressed to children and teenagers. These programs should contain the information of negative consequences of tobacco use, but what's even more important, preventive action designed specifically to address risk factors for children, adolescents and adults, especially parents and teachers. This task seems to be even more difficult taking into consideration all the factors mentioned above and additionally intensified target marketing run by tobacco corporations.

One of the basic aims of the prevention is to increase the knowledge about negative nicotine intake results. Most of the studies, including this report, revealed that knowledge seems to be sufficient in adults (up to 95% of them know the risk connected with cigarette smoking) [21] as well as in adolescents. In our study the proportion of examined students reporting the knowledge about negative smoking consequences reached 90.9%. The truth is that the knowledge about negative consequences of 'the' behaviour should be sufficient factor to stop it. However, the truth also is that human-beings can sometimes act illogically. Many authors have reported that knowledge of it's own was not the factor leading to change or stop negative behaviour [9,21,31]. To be successful one needs to realise the problem and to be motivated to change. On a social scale it requires financial means and combining a variety of effective actions, taking into account all factors affecting initiation or continuation of smoking.

Particularly dangerous is the early initiation of smoking which can be associated with wide range of negative health related consequences, higher risk of addiction as well as difficulties with cessation. Moreover, it has been documented that the symptoms of nicotine dependence in adolescents occur short after the smoking initiation, sooner than in adults and smoking may be a risk factor of other addictions [32]. Thus many authors highlight that prevention programs should be addressed to children as well as adolescents, especially due to the fact that cigarette smoking increases among children under 13 [12]. Research point out that probability of continuing smoking in adult life is higher among persons who started to smoke under 13 [33], according to other authors this tendency is observed in persons who initiate to smoke under 18 [12]. Thus, the delay of smoking initiation moment should become a vital aim of the prevention. The preventive interventions should also be focused on 18-year-old smokers considering themselves 'adults'. This group of adolescents is at higher risk of developing addiction in future. Previously reported parent-child relationship and its quality, parents' attitudes and behaviours (which can be preventive as well as risk factor) should be appreciated. As one of the studies revealed even smoking parents can effectively prevent smoking in their children [13].

It is worth noticing that teenagers rarely tend to initiate conversations with adults of risk behaviour [12]. It is, among others, connected with the fact that such behaviour is not approved of by them, often simply forbidden or being a taboo. The task for parents, tutors, psychologists and doctors is to get it through to children and adolescents with such arguments that will be understood an interesting for them. It is essential to avoid patronising which arises resistance irrespective of the importance of the problem. It is also important to match the form and contents of information with the converser/recipient's age as age, among others, determines the perception of reality. The same principle applies to anti-smoking campaigns and programmes. Threatening adolescents with distant consequences of smoking, such as: "If you smoke, in 20 years' time…" is an ineffective method. For young people less abstract, that is more probable, are immediate or not so distant negative effects of smoking. They will be more likely to accept such arguments while they will reject those not being very realistic to them. Form and contents should be adequate to young people's perspective so it would be positively perceived by a given age group and refer to important aspects of life of the group addressed.

In the context of pre-health or medical treatment actions taken, messages evoking negative emotions (fear, disgust), especially those using images, are more effective than neutral ones, provided they are properly used and include the method (strategy) of preventing or dealing with the threat. Stress should be rather put on positive aspects of not smoking than only on negative ones of smoking accentuating and focusing on advantages of not smoking seems to be more effective.

Taking into account a number of psychological factors favouring smoking, preventive actions should include methods

of developing children's and youth's personalities in such a way as to strengthen the qualities and teach the behaviour favouring abstinence. Developing assertive communication style, strengthening internal sense of control and self-efficacy, teaching the ability to reject negative social influence and indicating positive methods of coping with stress, increase sense of strength and enrich resources of activities useful in managing problems [5].

Attention should be paid to potential effectiveness of combining school activities with media campaigns, especially those aimed at youth mainly, as well as with open-air events (e.g. HELP European Campaign, Woodstock Station) and advertising banners [8]. Combination of such elements as information on imminent effects of smoking, positive effects of not smoking, education on social influence theory and training the ability to refuse, increases its effectiveness [12].

It is also worth noting that although universal activities addressed to large social group are doubtlessly valuable, the "individualisation" is also important. The basis of this idea are differences in temperament and personality traits, causes and factors favouring smoking related to age, gender, social and cultural factors. Taking these differences into account during anti-nicotine programmes preparation could result in their higher effectiveness.

Recapitulation

Prevention requires solutions in macroscale – covering whole country – and social campaigns, planned and developed for a concrete social group and aimed at that group. As Charlton et al. [8] suggest "non-smoking should become a norm" and school "a place free from cigarettes", also school employees should be banned smoking [8]. Survey performed in Poland in 2006 by PENTOR [34] showed that 76% of Poles supported ban on smoking in public places, large number being smokers themselves! Therefore even the persons to be affected by the ban are in favour of its introduction. Maybe such restrictions will make some smokers limit smoking or even quit the habit.

Percentage of smoking teenagers is still high, especially among vocational school girls, which has been confirmed by our study. Also lowering age of initiation of smoking and drinking alcohol is worrying. Further research aimed at finding causes and behaviour inducing smoking and other risk behaviour should be a valuable source of information when taking preventive and therapeutic activities. Those issues will be a subject of further research by the authors of this study.

Conclusions

1. Smoking is a big problem in population of 18-year-old students, irrespective of school profile.
2. It is alarming that 52.7% of women and 46.3% men in vocational schools smoke.
3. It is important to create educative programs not only in schools, dealing with ways of smoking prevention and fighting smoking habit in population on the verge of manhood and womanhood and in younger population.

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Bone structure regeneration after low induction magnetic field treatment in teeth chosen for extraction

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Abstract

Purpose: The aim of the work was to use and to evaluate the usefulness of the slow variable magnetic fields to aid the treatment of the teeth chosen for extraction. The marginal paradontium of periapical bone of teeth was in a state of extensive destruction. The teeth were chosen for extraction.

Material and methods: 13 patients were chosen. 10 of them had with endo-perio changes and 3 suffered from full tooth luxation and had the teeth replanted. Those people were to have an extraction procedure or were declared as impossible to treat in other dental offices. Patients underwent non-aggressive skaling, endodontic treatment and were exposed to slow variable magnetic fields generated by Viofor JPS, accordingly to methods and parameters suggested by Department of Propaedeutics in Dentistry of Pomeranian Medical University in Szczecin. The process of healing of changes was evaluated radiologically.

Results: RTG done after 2 weeks and after 2 months were evaluated in respect of bone regeneration. They show the bone structure concentration. A RTG evaluation after half a year, two and three years show a preservation of the bone structure concentration.

Conclusions: The use of slow variable magnetic fields contributed to bone structure regeneration and to preserve teeth with recorded endo-perio syndrome. Endodontic treatment of replanted teeth, aided with magnetostimulation has stopped the osteolisis process.

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Introduction

Nowadays stomatologists are seeking methods that help to preserve teeth chosen for extraction. It is due to the increase of patients' expectations, privatisation of stomatology, and tendency to preserve one's own teeth, despite the rise in implantology. Unequivocal reasons to tooth extraction are an extensive destruction of bone structure and considerable tooth luxation. In our work there was an attempt to use low variable magnetic fields in some teeth. They had endodontic-periodontic paradontium damages and traumatic – tooth dislocation. A preservative treatment was undertaken at patient's will. Those states are the biggest tooth damages and are often qualified to extraction [1]. Pathological endo-perio states that were due to changes in endodontium and periodontium are connected through apical gap and lateral canal. This is the reason why the healing of those chronic inflammatory states is a complex problem, which often results in a tooth extraction [2]. It is also difficult to gain good therapeutic result after a complete tooth luxation – a total loss of connection between a tooth and a dental alveolus. Such a tooth is usually dry-stored and kept long outside the oral cavity for more that 120 minutes.

Material and method

10 patients were chosen to endodontic treatment. They had endodontic-periodontic syndrome. It was identified with the use of clinical check-up, diagnostic tests (electric pulp vitality test) and radiological tests. The treatment was preceded by a non-aggressive skaling. After processing the canals they were filled with gutta-percha with AH Plus or Diaket. The roots were filled using one-cone or side condensation method. Since the first day of endodontic treatment, after eliminating possible contraindi-

Figure 1. **Tooth 41 – extensive apical and border osteolisis. At the beginning of treatment a surveying image was done with a needle. In the paraapical area on the RTG there is a large limited translucence. Around there is a bone calcification line. A bone septum between tooth 41 and tooth 31 has large bone holes. The rarefaction of the bone structure and irregular horizontal destruction of the alveolar, almost intermixed with an extensive periapical change are visible**

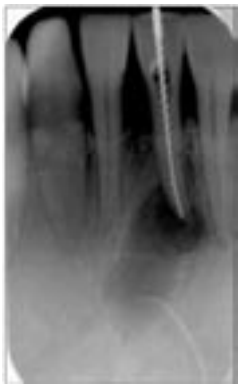


Figure 2. **Tooth 41. Status after two weeks of treatment together with magnetostimulation sessions. The tooth is filled with an iodoform substance that shows a direction of healing fistula in the periapical area. A fast rate of healing of the bone structure is visible. The diameter of the translucence of the periapical area became smaller. The alveolar bone became denser, what was confirmed with a use of Digora 2.1 programme**

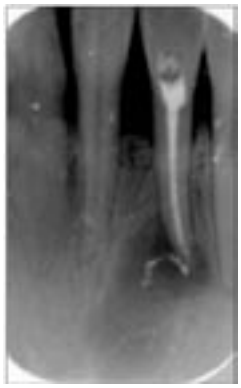


Figure 3. **Tooth 41. After two months of treatment and magnetostimulation a follow-up improvement is visible. The size of osteolisis has been reduced, and the bone structure has become denser. The root canal was filled with Diaket material**

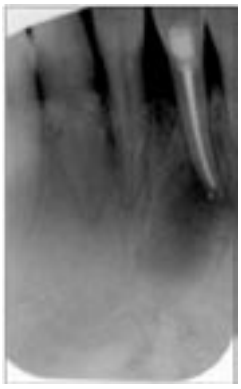
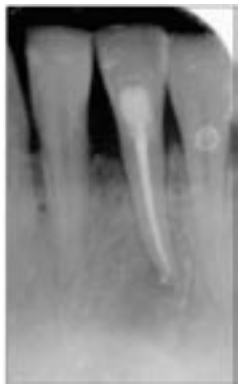


Figure 4. **Tooth 41. A control image done after half a year shows almost perfect recovery. The trabecular bone structure is completely rebuilt. A border line of the alveolar bone with a blurred border.**



cations, magnetostimulation treatment was applied with the use of Viofor JPS. A A3M1P3 programme was used. The initial 4 intensity was increased every three days, up to 6. The minimum number of sessions was 20. The technical details are in the Viofor JPS manual. A control RTG was done after 2 weeks, 2 months, half a year, 2 years and 3 years. Then it was evaluated with the use of Digora 2.1 system or on standard RTG.

The treatment of three patients with full tooth luxation (extraction) began. The time of seeking help and the way of keeping the tooth (dry) did not suggest preserving the tooth in a bone. After inserting the teeth into the dental alveoluses and fixing it, the magnetostimulation sessions has started accordingly to the method described above. Simultaneously, the teeth were treated endodonticly, accordingly to the method presented above. In case of the youngest patient (5 and a half year old) the endodontic treatment was not done. It was due to the unfinished development of the tooth root and to finding a broad bleeding neural-vascular bundle.

Results

Radiological description of the case

A.K. 45 years old patient with endodontic-periodontal syndrome. Due to extensity of changes her odds were low (see *Fig. 1-4*).

S.N. patient, 5 and a half year old, after a complete tooth 21 luxation and destruction of outer lamina of the alveolar bone. A Radiological presentation of a case and a bone structure regeneration process is presented in *Fig. 5-8*.

Discussion

The research on using the slow variable magnetic fields in stomatology was due to good results in treating inflammatory and degenerative states of movement organ and also traumatic states in general practice [4]. The research in stomatology continues for 5 years and is still continued [5]. Many mechanism of the influence of those fields on the tissue were recognised. In the Viofor JPS device the parameters of the induction of the fields are chosen in order to sustain the homeostasis of the organism. In case of the dysfunction of homeostasis the parameters allow to quickly regain the normal status. The electrodynamic, magnetomechanic influence and ion cyclotron resonance causes biophysical and biological effects in the organism [6]. It is believed that the magnetomechanic effect has an influence on bone matrix due to calcium reuptake and returning to bone. Some of the examples of the influence of the fields on the molecular level are the activation of the enzymatic reactions, the capacity enhancement of the cell membrane and the influence on the movement of electric charges. It is believed that the intensification of an osteogenic process is connected with vasodilatative and angiogenetic effect, which enhances process of oxygenation and nourishing tissues [7].

Extensive destruction of the side paradontium and apical

Figure 5. **The status at the beginning of treatment – 6 hours after the injury. The tooth was dry-preserved in xylogen.**
On the RTG an empty dental alveolus of the knocked out tooth 21. The damage of outer lamina of alveolar bone (break) is matching to the length of the developing root. Next there is a tooth 11 with unfinished development of the tooth – large latitude of the root canal

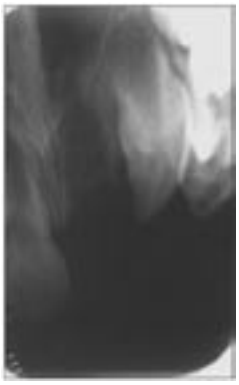


Figure 6. **After two weeks after immobilising the tooth and using magnetostimulation sessions the tooth integrates with a bone in the root apical area. The teeth are joined with a use Splint-it tape**

Figure 7. **RTG after two months from the replantation. Status after taking of the splint. The contact between tooth root and bone is minimal. A high risk of loosing the tooth**

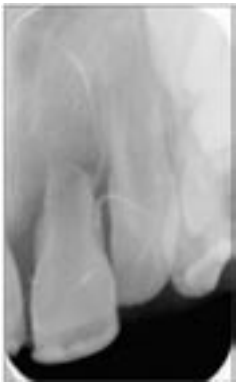
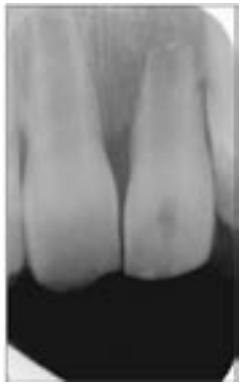


Figure 8. **RTG after 4 months from injury. The root of teeth 21 does not grow lengthwise. There are no symptoms of root resorption. The line of the tooth root apes calcification is noticeable. There is an alveolar bone regeneration and the root is fusing with the bone**



structure are still difficult dental problems. A decision of not removing a tooth was caused by the tooth localisation, patient's hygienic status, his or her attitude towards therapy, age and financial status.

An endoosteum localisation of the paraapical osteolisis and their structure prevents the antibiotic to reach its aim. This is why the antibiotic therapy was unsuccessful in that cases [8]. However, in endo-perio changes, especially II and III type, there is a need to give an antibiotic.

In the given clinical material the endodontic-periodontic treatment, aided with magnetostimulation was implemented after diagnostics. Among the results of the treatment were good effects in stopping the process of paradontium and apical destruction and stopping or slowing the process of root resorption and the osteolisis of the border bone tissue.

A positive influence of the magnetic fields on the intensification of the osteogenic process, even in cases of extensive osteolisis, was observed. The influence on the piezoelectric and magnetostrictic structures, such as collagen, dentine or keratin, leads to enzymatic transformation's intensification and to calcification of the bone structure [9].

Conclusions

1. The used magnetostimulation method contributes to thickening of bone structure and is very useful in healing the endodontic-periodontic syndromes.

2. The use of slow variable magnetic field with low induction slowers or stops the begun process of bone osteolysis after replantation.

3. The use of low induction magnetic fields allows the patient to keep his or her own tooth for many years despite of unfavourable prognosis. The treatment confirmation is gained in this way.

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New technology in endodontics – the Resilon-Epiphany system for obturation of root canals

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Abstract

Purpose: **Clinical and laboratory assessment of a new root canal filling material – Resilon-Epiphany system.**

Material and methods: **In 21 patients, 48 root canals were filled using a single-cone method or lateral condensation technique of gutta-percha with addition of Epiphany sealer. Laboratory investigations were performed on 4 extracted one-root human teeth, which were prepared by means of a crown-down technique and obturated with Resilon-Epiphany using System B and Obtura II. Next, the roots were transversely cross-sectioned in the mid-length at a 2 mm distance from the apex and analysed in SEM.**

Results: **After a year, the treatment proved to be clinically and radiologically successful in all the patients. SEM analyses revealed good adhesion of Epiphany sealer to the canal walls with visible tags in dentine tubules. Good adherence was also found of Epiphany to Resilon and Resilon to root dentine, but few gaps were also observed.**

Conclusions: **Our preliminary positive results require more thorough evaluation, longer observation period and a larger group of patients. However, they allow the assumption that resin-percha will successfully replace gutta-percha in the nearest future.**

Key words: Resilon, endodontic treatment, seal of root canal obturation.

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Introduction

Success in endodontic treatment is predominantly determined by complete obturation of the canal system. Gutta-percha, which has been commonly used for this purpose, does not prevent bacterial leakage and further complications, even when applied together with a sealer [1-4]. Thus, finding a gutta-percha substitute that would provide a superior seal of the root canal system has become a challenge in modern endodontics. A new material, the Resilon-Epiphany system with its novel formula, may revolutionize endodontic treatment (*Tab. 1*). The system consists of three parts: Resilon – a thermoplastic synthetic polymer-based (polyester) root canal filling material, as the major component; Epiphany sealer – a resin-based composite that forms a bond to the dentin wall and the core material under chemical reactions and halogen curing light; and Primer, which prepares the canal wall to get in contact with Resilon and the sealer [5-7]. Resilon looks and handles like gutta-percha and is therefore called resin-percha [8]. It is available in standardized points that fit endodontic instruments and in various tapers, as well as in accessory points and pellets for use with the Obtura II delivery system. Various techniques can be employed to place this material in the canal (single-cone method, cold lateral condensation and thermoplastic techniques), with the same instruments and devices that are used for gutta-percha condensation [9,10].

Based on composite resins, the Resilon-Epiphany system is a new generation material used so far for cavity restoration in the coronal hard tissues. Endodontically applied, this system allows formation of the so called mono-block made of root dentine, sealer and resin-percha [5-7], which has the potential to strengthen the structure of the tooth attenuated by endodontic treatment [7], at the same time ensuring complete sealing of the root canal, resistant to bacterial leakage [9,10].

The aim of the study was to present clinical and laboratory assessment of a new root canal filling material – the Resilon-Epiphany system.

Table 1. **Composition of Resilon System**

Resilon core material organic part: thermoplastic synthetic polymer – polycaprolactone, inorganic part: bioactive glass, bismuth oxychloride, barium sulphate
Resilon Sealer organic part: BisGMA, ethoxylated BisGMA, UDMA, hydrophilic difunctional methacrylates inorganic part: calcium hydroxide, barium sulphate, barium glass, bismuth oxychloride, silica
Resilon Primer sulfonic acid terminated functional monomer, HEMA, water, polmerization initiator

Material and methods

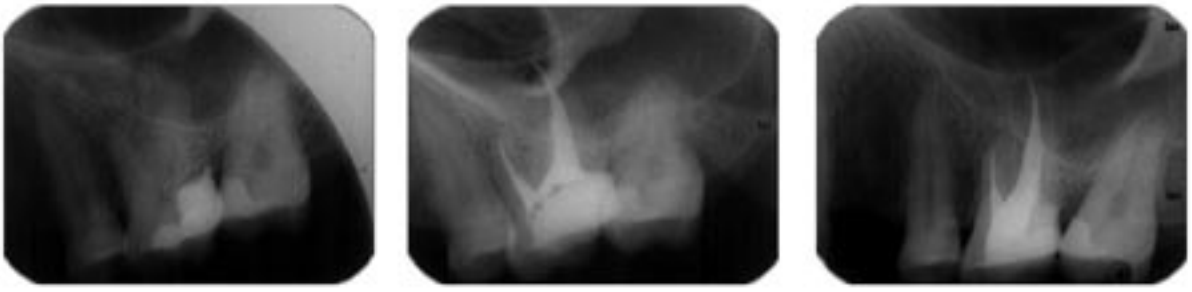
Twenty-one patients (16 women and 5 men) aged 14-55, with endodontically treated 48 root canals in 24 teeth (5 incisors, 1 canine, 6 premolars and 12 molars) were enrolled in the study. The patients' history, as well as dental and radiological examinations revealed irreversible pulpitis with vital pulp in 11 cases, acute apical periodontitis in 2 cases, chronic apical periodontitis in 5 cases (granulous in 4 and suppurative in 1), and exacerbated apical periodontitis with submucous abscess in 2 cases. Four teeth with vital pulp were treated for periodontal reasons, prior to periodontal surgery. Pulpectomy was performed in 15 teeth: in local anaesthesia (8) or after intentional devitalization (7). In the remaining cases, antiseptic root canal treatment was instituted with the use of calcium hydroxide nonsetting paste – Biopulp, placed in the canal with a Lentulo spiral for 7-21 days. In two patients with submucous abscess, abscess was incised, and the patients were given Dalacin C at a dose of 0.15 g every 6 hours and Metronidasol at a dose of 0.25 g every 8 hours. "The tooth left-open treatment" was not employed.

When radiographs were taken, the teeth were isolated from the saliva and the coronal cavity was prepared to allow access to root canals. Then, the canal orificies were prepared with Gates Glidden drills and barbed broaches or files were used to remove the canal contents. Working length of the root canal was established by means of an electronic apex locator Raypex 4 (Morita). Root canals were prepared manually to obtain the size of master apical file (30-40), by means of the step-back method with K-reamers and nickel-titanium S-files (Poldent) or by means of a crown-down technique with nickel-titanium rotary files Hero 642 (MicroMega) to the level of dentinocemental junction. During instrumentation, the canals were irrigated with 1% sodium hypochlorite solution, 17% sodium versenate and distilled water applied with a syringe and needle with lateral opening. Then, they were dried with sterile paper points. When extirpated in anaesthesia, the canals were filled immediately after instrumentation or on the subsequent visit. The infected canals were closed during the second and third visit. Resilon points and Epiphany sealer (Pentron), used to fill the canals, were inserted by means of a cold lateral condensation technique (for hand preparation) or by a single-cone method with grater-taper points (for rotary instrumentation). Prior to resin-percha point application, the canal walls were coated with a self-etching primer (Epiphany primer) placed for 30 sec. with a special brush included in the set. Primer excess was removed with paper points. In the lateral condensation technique, a premeasured master point to fit the diameter and length of the root canal, coated with the sealer (Epiphany Root Canal Sealant) was placed in the root canal and condensed with a finger spreader. The rest of the canal was filled up with accessory points dipped in a small amount of sealer, which was halogen light cured for 40 seconds. When the single-cone method was used, the sealer was applied to the canal with a Lentulo spiral (Poldent), and then a suitable Resilon cone (04) was placed to working length. When the canals were filled, the excess points were removed with a hot instrument and a control radiograph was taken. Phosphate cement Agatos was inserted into the chamber and a composite material Herculite XR (Kerr) was placed in the cavity.

After the canals had been filled, as well as 6 and 12 months after treatment, we examined the patients for spontaneous and biting pain, tenderness to palpation of the alveolar process in the projection of the root apexes of the treated teeth, periapical tissue reaction to vertical and horizontal percussion, and crown colour. Radiographs were analysed for the quality of obturation (tightness, the level of filling from the radiographic apex), periapical status (the width of periodontal ligament space, lamina dura continuity around the apex, structure of the alveolar bone in the periapical region).

Laboratory investigations evaluating the tightness of the seal of the obturation were performed on 4 extracted one-root and one-canal human teeth, which were stored after extraction in 1% sodium hypochlorite solution. The crowns were cut off at the level of the cementoenamel junction with a high-speed diamond bur. Then, the canal orificies were prepareded with a Gates Glidden drill and a barbed broach was used to remove the pulp. The working length of the root canal was established at 1 mm short of the anatomical apex. Root canals were prepared using the crown-down technique with nickel-titanium rotary instruments Hero 642 (MicroMega) to obtain the 30/40 size of the master apical file. During instrumentation, the canals were rinsed with 1% sodium hypochlorite, 17% sodium versenate and distilled water applied with a syringe and needle with lateral opening. Then, they were dried with sterile paper points and filled with the Resilon-Epiphany system. Prior to the application of the obturative system, the canal walls were coated with a self etching primer, as above; 1/3 of the periapical part of the root was filled with a suitable Resilon point, using the continuous wave method and System B (SybronEndo). A small amount of Epiphany sealer was placed into the root canal with point. Backfilling was performed with Obtura II (Obtura Corp.) using 25 gauge needle tips at a temperature of 160°. Epiphany sealer was not light-cured. Control radiograph was taken and the chamber was closed with phosphate. The roots were stored at 37° C for 7 days, in hermetic vials containing cotton-wool swabs soaked with distilled water. Then, the roots were cross-sectioned transversely at the mid-length and 2 mm from the anatomical apex (8 samples) [11]. Sectioned root fragments were embedded in aluminium rings filled up with epoxy resin. The images were analysed in a scanning electron microscope SEM Hitachi S-300 (Hitachi), without coating in low-vacuum conditions (40 Pa) [12]. The sealer adherence to resin-percha and root dentin, as

Figure 1. **Patient RTG, 36 years old, tooth 26, diagnosis – pulpopathia irreversibile**



a) Radiograph before treatment

b) Radiograph directly before root canal filling

c) Radiograph a year after treatment

Figure 2. **Transverse cross-section of the root canal obturated with Resilon-Epiphany system. Sealer (U) adheres tightly to dentine (Z) and Resilon (R), sealer tags are visible in dentine tubules (arrow). Magnification 3000x**

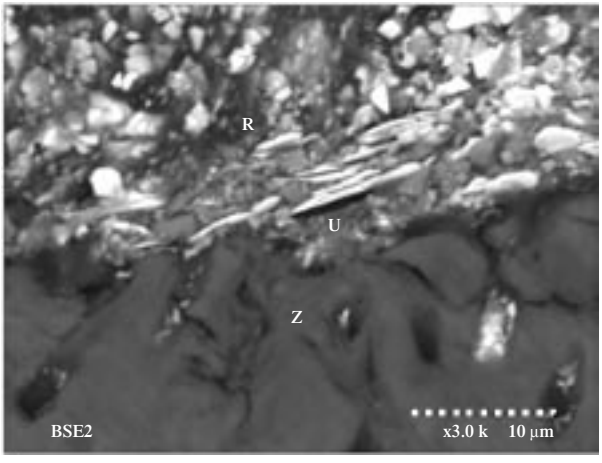
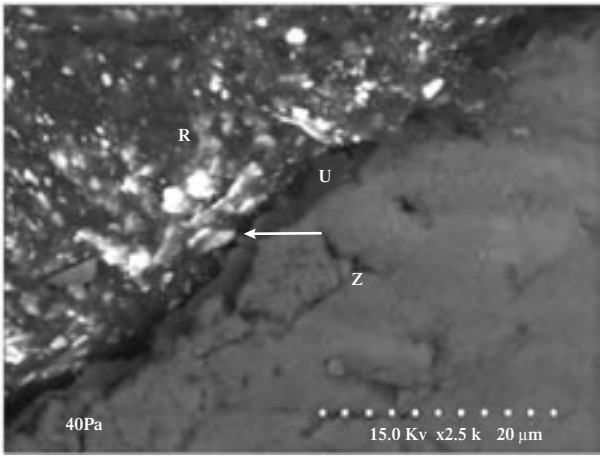


Figure 3. **Transverse cross-section of the root canal obturated with Resilon-Epiphany system: a) A 1,2 μm wide gap visible between sealer (U) and Resilon (R) (arrow). Magnification 2 500x; b) Visible is the sealer (U) adhering to dentine (Z) as well as gaps between the sealer and Resilon (R) (arrows), a likely result of root cutting. Magnification 3 000x**

a)



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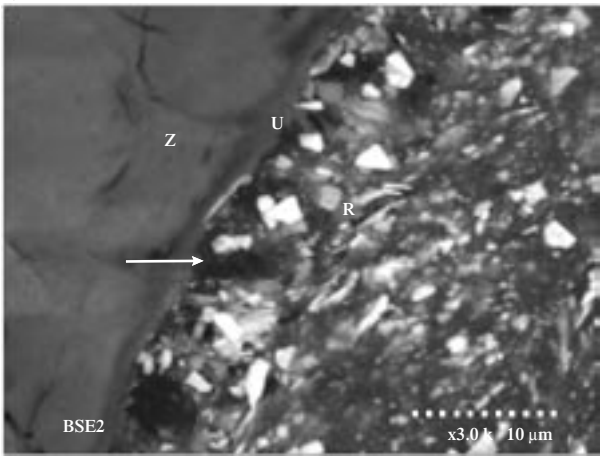

well as bonding of resin-percha to the canal walls were evaluated. The gap width between the respective elements of the filling and dentine was recorded.

Results

A. Clinical evaluation

Treatment results have been presented in *Fig. 1a*, *1b* and *1c*. Directly after filling insertion, in four cases with radiologically confirmed root canal overfilling, we observed pain on biting and positive reaction of periapical tissues to vertical percussion. The symptoms subsided after 2-3 days. In the remaining cases, the canals were properly filled (0-2 mm filling distance from the radiographic apex) and no pain was reported either after the fillings were inserted or 6-12 months after treatment termination. No clinical crown discolouration was noted throughout the observation period. All the follow-up radiographs showed normal picture of the periapical structures. Bone regeneration at the site of bone loss was observed in teeth with chronic and exacerbated apical periodontitis.

B. Laboratory evaluation

The results have been presented in *Fig. 2-3a, b*. The transverse cross-section pictures of the periapical and pericoronal margins were recorded. All the samples exhibited very good adhesion of the sealer to the dentine and resin-percha. In

large magnifications (1500x, 3000x) sealer tags were present in dentine tubules (*Fig. 2*). Sporadically, single gaps, 1.2-6 μm wide, were noted between the sealer and Resilon (*Fig. 3a, b*). Moreover, in four sites of the analysed pictures (2 samples), sealer-free, uneven Resilon adherence to dentine and 0.75-8 μm gaps could be seen.

Discussion

According to the producer, the unique properties of the Resilon-Epiphany system allow the formation of the so called mono-block with root canal walls – the sealer adheres to Resilon points and root dentine, properly prepared by means of the primer. This is expected to ensure a complete hermetic canal filling seal reducing bacterial leakage [5].

In all the current study cases, we observed a positive outcome 6-12 months after treatment termination. As no literature reports are available on the clinical assessment of Resilon, we were not able to compare the present results. Shipper et al. [10] evaluated the efficacy of Resilon and Epiphany in comparison to gutta-percha and AH 26 pasta in the prevention of apical periodontitis. They created conditions for reinfection of the previously filled root canal system and found a significantly lower rate of apical periodontitis in resin-percha filled teeth. The authors suggest that Resilon and Epiphany have better sealing properties and thus greater resistance to bacterial leakage as compared to gutta-percha, the finding which has been also confirmed by other studies [5,9].

The laboratory examinations revealed very good, gap-free adherence of sealers to dentine, both in the periapical and coronal root fragments. Single gaps were seen between Resilon and Epiphany, as well as between Resilon and dentin. Similar results have been reported by Tay et al. [13], who in SEM compared the tightness of root canal obturation with the Resilon-Epiphany system and gutta-percha and AH Plus pasta, using System B and Obtura II. In both groups, the authors observed both gap-free and gap-containing regions. It is assumed that these gaps are probably created by rapid polymerization contraction, promoted by heat generated during material condensation with a hot plugger. Additional manipulations in the course of further insertion of the material to the canal damage the bonds between the respective elements of the filling and root dentine [13-15]. Other causes of gap formation may include manipulations during sample preparation for SEM examinations i.e. root cutting, placement in vacuum, dehydration prior to coating [16,17]. In our study, some gaps had contours corresponding in shape and size to the filler particles that were probably pulled out during root cutting (*Fig. 3 b*)

Conclusions

The present evaluation of the Resilon-Epiphany system has yielded positive outcome both in clinical and microscopic examinations. However, these preliminary results require more profound analysis, longer follow-up period and a larger number of patients. Nevertheless, we can assume that resin-percha will successfully replace gutta-percha in the nearest future.

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Assessment of salivary levels of the chosen exoglycosidases in patients with aggressive periodontitis after treatment with doxycycline

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Abstract

Purpose: **The aim of the study was the clinical assessment of the periodontium in patients with aggressive periodontitis (AP) after treatment with doxycycline hyclate. Moreover, an attempt was made to evaluate the effect of the treatment on the salivary concentrations of β-glucuronidase, HEX, HEX A and HEX B in AP patients.**

Material and methods: **Sixteen patients with aggressive periodontitis, aged 28-45 years, were enrolled in the study. The patients were treated with a doxycycline hyclate preparation (Periostat) for 2 months at a dose of 20 mg twice a day. The clinical examination was performed twice, directly prior to pharmacological treatment and after its termination. The following clinical parameters were evaluated: the plaque index (PI), the sulcus bleeding index (SBI), the pocket probing depth (PPD) and the clinical attachment level (CAL). Biochemical determination of β-glucuronidase, HEX, HEX A and HEX B concentrations in non-stimulated saliva was performed before and after treatment.**

Results: **In AP patients, the values of PI, SBI and CAL before and after treatment were comparable. The mean pocket probing depth before treatment was 3.5 mm, which decreased significantly after treatment (3.2 mm). The values expressed as pKat/kg protein for specific enzymatic activities of HEX, HEX A, HEX B and β-glucuronidase in the saliva of AP patients before and after doxycycline treatment were similar.**

Conclusions: **A 2-month treatment with doxycycline is too short to obtain clinical changes. Although the assessment of the activity of such enzymes as β-glucuronidase, HEX, HEX A and HEX B in the saliva of AP patients allows detection of periodontal inflammation, it cannot be used to determine the risk of its development and therefore has no practical significance.**

Key words: aggressive periodontitis, doxycycline hyclate, proteolytic enzymes.

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Introduction

Periodontal inflammations are progressive diseases of the tooth supporting structures [1]. Their pathogenesis is very complex, with dental plaque being the major etiologic factor. Over 500 species of bacteria have been identified in dental plaque but only several of them, especially *Actinobacillus actinomycetemcomitans* and the red complex bacteria cause periodontal tissue destruction [2]. Due to inflammatory reactions, numerous proteolytic enzymes which destroy matrix proteoglycans are released [3,4]. These enzymes belong to the class of exoglycosidases and include N-acetyl-β-hexosaminidase (HEX), β-galactosidase, α-mannosidase, α-fucosidase and sialidase, which split single monosaccharides off the non-reductive oligosaccharide terminal portion and are specific for one anomeric form of glycosyde bond. Together with endoglycosidases they form a series of reactions, in which the product of one reaction is the substrate of the subsequent one [5].

N-acetyl-β-hexosaminidase (HEX, NAG, E.C. 3.2.1.52) is the most active lysosomal enzyme. It hydrolyses saccharose chains of glycoconjugates, releases N-acetyloglucosamines and N-acetylogalactosamines from various β-oligosaccharides of glycopeptides and glycoproteids, and during hyaluronic acid breakdown [6]. The presence of this enzyme has been found in the saliva, blood serum and plasma, the cerebrospinal fluid and articular fluid, as well as in many tissues and organs, e.g. in animal salivary glands [6-9]. It has been proved that HEX is produced in mucous and epithelial cells of outlet ducts in the sub-

Table 1. **Clinical parametres (mean, ±standard deviation) in preeliminary and follow-up examination**

Parameter	Examination I	Examination II
PI	0.7±0.44	0.8±0.49*
SBI	43.1±17.39	46.3±18.48
PPD	3.5±0.77	3.1±0.67*
CAL	4.2±1.23	4.0±1.1

* – statistical difference between I and II examinations; PI – plaque index; SBI – sulcus bleeding index; PPD – periodontal pocket depth; CAL – clinical attachment level

Table 2. **Specific activity pKat/kg exoglycolidase protein (mean, ±standard deviation) in preeliminary and follow-up examination**

Exoglycolidase	Examination I	Examination II
HEX	10.8±3.91	13.3±4.27
HEX A	6.8±2.62	6.9±2.95
HEX B	4.1±2.09	6.4±5.41
β-glucuronidase	4.2±2.27	5.0±1.52

mandibular salivary gland [8]. HEX is built of two polypeptide chains – α and β. A few N-acetyl-β-hexosaminidase isoenzymes have been isolated: A, B, C, I_1, I_2, P, S. HEX A contains α and β chains, HEX B and P have two β chains. Immunoenzymatic tests using anti-HEX B antibodies have allowed classification of isoenzymes B, I_1, I_2, P as one HEX B group [5].

β-glucuronidase (glucoronohydrolase of β-D-glucuronide E.C. 3.2.1.31) is responsible for the reaction that yields β-glicuronians – compounds of the glucuronic acid with phenol, alcohols and carboxy acids. Formation of such conjugates is a known method of detoxication. β-glucuronidase has been found in the secretion of parotid and submandibular glands [10]. Elevated levels of N-acetyl-β-hexosaminidase and β-glucuronidase have been observed in the gingival sulcus fluid, saliva and periodontal tissues of patients with periodontal disease [3,4,11-16].

In order to inhibit the disease and stabilize the attachment level, the periodontal treatment aims to decrease periodontal pocket pathogens [17]. It consists of three phases: preliminary, corrective and supportive. In the latter, the mechanical procedure reducing the number of bacteria is complemented with general pharmacotherapy, which can be either addressed against periodontal pocket pathogens or modulate the host response. The drug used to modulate the host response is doxycycline hyclate, which affects local inflammatory reactions through the release of enzymes, metalloproteinases (MMP) in the first place [18]. Administration of doxycycline to periodontitis patients caused flattening of the periodontal pocket depth (PPD), reduction in the clinical attachment (CAL) and decreased bleeding (SBI) [1,11,19-23].

Therefore, an attempt was made to clinically assess the periodontal status of patients with aggressive periodontitis (AP) after treatment with doxycycline hyclate. Moreover, we decided to evaluate the effect of the treatment on the salivary concentrations of β-glucuronidase, HEX, HEX A and HEX B in AP patients. Changes in the levels of these enzymes could be potentially used as inflammation reduction indices and serve as prognostic markers of the disease.

Material and methods

The study involved 16 patients with aggressive periodontitis, aged 28-45 years (10 women and 6 men). A few weeks before the start of the treatment all the patients underwent professional dental cleaning. Then, they were treated with a doxycyline hyclate-containing preparation (Periostat, CollaGenex, USA) for 2 months at a dose of 20 mg twice a day. The preliminary examination was performed directly before, while the check-up after the pharmacological treatment. A periodontal probe PCP 11 (Hu-Friedy, Finland) was used for examinations.

The following parameters were used for clinical assessment of the periodontium:

- the plaque index (PI) according to Silness and Löe [24],
- the sulcus bleeding index (SBI),
- the pocket probing depth (PPD) (in mm),
- the clinical attachment level (CAL) (in mm).

The biochemical methods used to determine the levels of β-glucuronidase, HEX, HEX A and HEX B in the non-stimulated saliva included:

- N-acetyl-β-hexosaminidase and its isoenzymes A and B – the method of Chatteriee et al. [25], as modified by Zwierz et al. [26], Department of Pharmaceutical Biochemistry, Medical University of Białystok,
- β-glucuronidase – the p-nitrophenolic method [27], in own modification, Department Pharmaceutical Biochemistry, Medical University of Białystok,
- Protein level was determined with Lowry method [28], Department of Pharmaceutical Biochemistry, Medical University of Białystok.

A packet SPSS 8.0 PL was used for statistical analysis of the results. The t-Student test for pairs was applied to compare changes in the parameters at time intervals in the respective groups. Differences were considered statistically significant for $p \leq 0.05$.

Results

In the study group of AP patients the PI and SBI values obtained before and after treatment with Periostat were comparable. The mean depth of periodontal pockets before treatment was 3.5 mm and decreased significantly after treatment to 3.2 mm ($p=0.0009$). The mean CAL value after treatment did not change. The numerical values for these clinical parameters have been listed in *Tab. 1*. The values expressed as pKat/kg protein for specific enzymatic activities of HEX, HEX A, HEX B and β-glucuronidase in the saliva of AP patients before and after doxycycline treatment were similar. The mean values of the enzymes and standard deviations have been presented in *Tab. 2* and *Fig. 1-4*.

Figure 1. **Specific activity pKad/kg protein HEX before and after treatment**

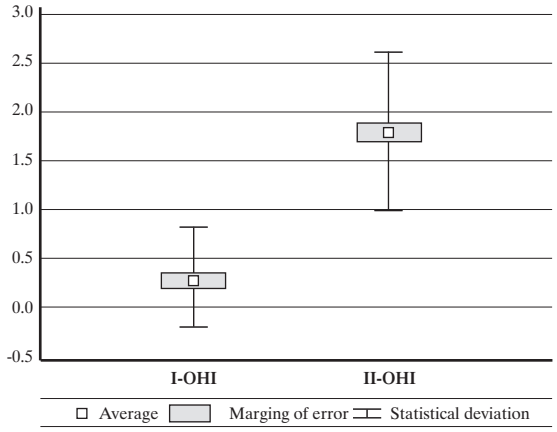


Figure 2. **Specific activity pKad/kg protein HEX A before and after treatment**

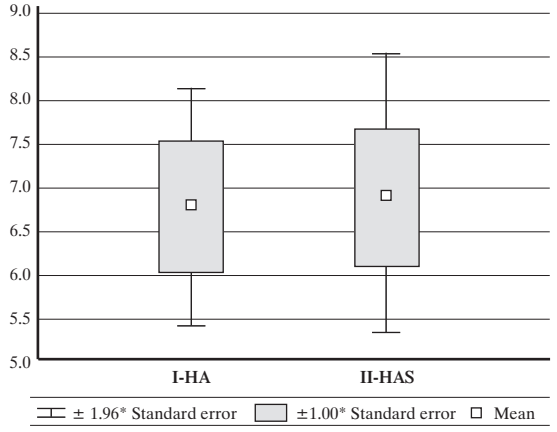


Figure 3. **Specific activity pKad/kg protein HEX B before and after treatment**

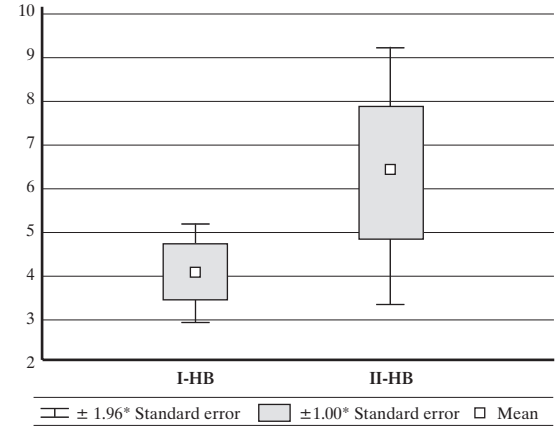
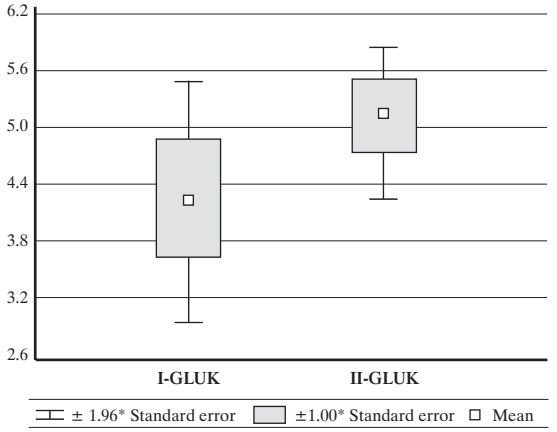


Figure 4. **Specific activity pKad/kg protein β-glucuronidase before and after treatment**



Discussion

Proper scaling, root planning and hygienic regime followed by patients are the standards of periodontal treatment. In 1998, periodontal therapy was complemented with doxycycline hyclate – a drug for use in combination with scaling and root planning [18,19,22]. It has been shown that a low 20 mg dose of doxycycline reduces the inflammatory process without undesired side-effects, i.e. it does not induce excessive growth of opportunistic flora, does not change bacterial sensitivity to antibiotics or induce resistance of bacteria in periodontal pockets [1,11,17,18,20-23]. The drug produces no side-effects, except for slight transitory gastric disorders, which are statistically insignificant as compared to the control group. This was also observed in our own material [21].

In the current study, no significant changes were found in the majority of the clinical parameters after doxycycline treatment. Statistically significantly reduced was only the pocket probing depth. Lack of differences in the clinical parameters can be explained by the fact that the patients were treated with the preparation for a short time, only for 2 months. Other authors have assessed the periodontal status after longer treatment with doxycycline hyclate. Ciancio et al. [21] showed considerable improvement in CAL, PPD and SBI after 12 months of treatment, which according to the author may be caused by the inhibition of the release of the enzymes that damage collagen. The improvement in SBI is probably associated with better cohesion of collagen structure and not with anti-inflammatory effect of doxycycline. Long-term studies conducted by other authors have also demonstrated improvement in the clinical parameters [1,19,29]. However, reductions in PPD and CAL have been found to be greater in periodontal pockets that are at least 7 mm deep [11]. In our group of patients the mean PPD was 3.5 mm, with the highest PPD value being 4.7 mm, and hence the changes after treatment were insignificant.

The diagnosis of periodontal inflammations is based on the clinical and radiological examinations. However, as various inflammations show different activities, some attempts have been made to institute a number of differential diagnosis tests that would facilitate the disease prognosis by assessing the levels of various enzymes in the periodontal pocket. However, due to high price the test have not come into wide practical use. The assumption of the current study was the analysis of changes in the levels of the chosen enzymes: β-glucuronidase and N-acetyl-β--hexosaminidase in the saliva of AP patients after pharmacological treatment. These enzymes are present in the granules of primary neutrophils, whose migration to periodontal tissues and gingival sulcus is a particularly important consequence of dental

plaque accumulation [3,15]. The mean salivary enzyme levels did not change, although the analysis of the respective cases revealed an increase in the concentrations of β-glucuronidase and N-acetyl-β-hexosaminidase in 12 patients, while a decrease in 4. The increase in salivary enzymes in periodontitis patients can be caused by a number of factors and may occur despite the pharmacological treatment instituted. Lack of proper hygienic regime, which seems to be the most important, is associated with the accumulation of dental plaque and thus with PI increase. The increase in β-glucuronidase positively correlated with the presence of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema denticola*, i.e. the main components of the subgingival plaque [13,16].

Specificity and sensitivity are the major features of any diagnostic test. There are various levels of specificity and sensitivity for various β-glucuronidase values, e.g. the analysis show specificity of 32.2% and sensitivity of 91.3% for 40 units, and 84.9% and 42%, respectively, for 100 units. Therefore, some patients with poorly pronounced inflammatory changes can have high levels of β-glucuronidase, while those with advanced periodontitis – low values of this enzyme [4]. The study outcome can also be affected by contamination with blood, being an additional source of the enzymes [14]. According to some authors, the most intensive enzymatic growth is observed at the sites of the most severe inflammatory symptoms, where PPD is >5 mm [14,16]. Nieminen et al. [12] did not observe a statistically significant decrease in the concentration of three salivary exoglycosidases: β-HEX, β-galactosidase and α-glucosidase after 9 months of periodontal treatment [12]. Our current study was conducted on a group of patients previously subjected to periodontal treatment. It can be thus assumed that tests detecting β-glucuronidase can be useful in patients never before treated for periodontitis as well as in patients with gingivitis which may progress and develop into periodontitis [15]. Thus, the assessment of enzymatic activity allows only detection of inflammatory periodontal changes, which has no practical advantage.

Since the tests used to evaluate the salivary levels of exoglycolidases: β-glucuronidase, HEX, HEX A and HEX B vary in specificity and sensitivity and the results differ according to the disease advancement, they cannot be used as prognostic tests to determine the risk of the disease development.

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Efficacy of local treatment with chlorhexidine gluconate drugs on the clinical status of periodontium in chronic periodontitis patients

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Abstract

Purpose: Chlorhexidine gluconate is a relatively commonly used chemotherapeutic in the treatment of periodontitis (P), exhibiting antimicrobial capabilities against Gram-negative and Gram-positive bacteria, and fungi. This compound is a component of various preparations for topical use in the form of solutions for mouthrinsing or peri-irrigation, gels, varnishes, chips and even chewing gums. The aim of the study was the clinical evaluation of periodontium after treatment with one of the drugs containing chlorhexidine gluconate (Corsodyl) as compared to professional tooth cleaning in patients with chronic periodontitis.

Materal and methods: Forty subjects enrolled in the study were divided into four groups, 10 in each group, according to the mode of treatment (Corsodyl rinse, Corsodyl gel, Corsodyl gel + surgical dressing, scaling).

Results: The greatest differences between baseline and follow-up examinations were observed in the group where surgical dressing was applied in addition to Corsodyl gel and in the group treated with scaling.

Conclusions: Chlorhexidine gluconate should be more frequently used as a drug adjunct to classic periodontal therapy, especially in the forms allowing its direct application to the periodontal pockets.

Key words: chlorhexidine gluconate, chronic periodontitis.

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Introduction

Chronic periodontitis (CP) is a common ailment affecting adult humans. Its main aetiological factor is the bacterial plaque accumulating on the tooth surface due to hygienic neglect. The effective methods, commonly used to eliminate dental plaque, include scaling with root planing and periodontal surgical procedures. Obviously, appropriate plaque control following professional mechanical cleaning of root surfaces is indispensable for the disease inhibition [1-3]. Such a control involving individual hygienic procedures is possible in many patients. However, there are a number of subjects who, for mental or manual reasons, are incapable to comply with the appropriate hygienic standards to maintain the effects of treatment and to prevent the disease recurrence. It is in these patients that the use of chemotherapeutics in combination with traditional therapy can help prevent the recolonization of pathogenic bacteria in periodontal pockets.

Chlorhexidine gluconate is a safe, recognized and more frequently used chemotherapeutic in the treatment of periodontitis (P), exhibiting an action against Gram-negative and Gram-positive bacteria, and fungi [4,5]. It is a component of various preparations for topical use, such as solutions for mouthrinsing or perio-irrigation, gels, varnishes, local delivery systems (PerioChip), and even chewing gums [2,5-9].

The aim of this study was the clinical assessment of the periodontium after treatment with a chlorhexidine digluconate preparation (Corsodyl) in comparison to the procedure of professional tooth cleaning in subjects with chronic periodontitis.

Material and methods

Forty patients with CP, aged 30-65 years (17 women and 23 men), were enrolled in the study. All the patients underwent scaling and root planing. Then, they were divided into four groups, 10 in each group, depending on the treatment applied. Group I included patients who rinsed the oral cavity with 0.2% solution of chlorhexidine digluconate for one minute (Corsodyl,

Table 1. **Assessment of clinical parameters after application of Corsodyl fluid with regard to periodontal pocket depth**

Parameter	Group I (< 5 mm)			Group II (≥5 mm)		
	Examination			Examination		
	I	II	III	I	II	III
PI	2.0±0.58	1.0±.58* p=0.01	0.4±0.53** p=0.01	2.3±0.66	1.3±0.66* p=0.0000	0.6±0.49** p=0.0000
SBI	2.7±0.76	1.9±0.90* p=0.04	1.3±0.49** p=0.01	3.0±0.88	2.5±0.75* p=0.001	1.3±0.45** p=0.0000
GI	2.0±0.58	1.4±0.53	1.1±0.38** p=0.04	2.3±0.66	1.8±0.36* p=0.003	1.3±0.45** p=0.0003
Clinical attachment level	3.0±1.27	2.8±1.17	2.8±1.03	7.1±1.69	6.7±1.73* p=0.009	6.6±1.80** p=0.001
Pocket depth	3.0±0.36	2.9±0.5* p=0.04	2.8±0.54** p=0.03	5.8±1.14	5.3±1.29* p=0.007	5.3±1.24** p=0.001

* – statistically significant difference between examination I and II; ** – statistically significant difference between examination I and III

GlaxoSmithKline) twice a day for three weeks. Group II consisted of patients treated with 1% Corsodyl gel (GlaxoSmithKline) applied to periodontal pockets at one-week intervals. In group III, the treatment was the same as in group II, but in order to delimit drug leaking from periodontal pockets and its dissolving in the saliva, adhesive surgical dressing Reso-Pack (Meyer Haake) was used to seal the teeth and the surrounding soft tissues and was kept in the mouth for several hours subject to gradual dissolving. In group IV (control), no pharmacological treatment was instituted.

Clinical examinations were carried out three times by the same person with the use of a periodontal probe PCP 11 (LM Dental). The preliminary examination in the first three groups had place a week after scaling, directly before application of the drug. The other two took place one month and three months after the first. In the control group, the first examination was performed before scaling, the other two – one month and three months later. Clinical examinations were based on the assessment of the following parameters:

- PI (Plaque Index) accrding to Silness and Löe [10]
- SBI (Sulcus Bleeding Index) accrding to Mühlemann and Sonn [11]
- GI (Gingival Index) accrding to Löe and Silness [12]
- periodontal pocket depth (in mm)
- clinical attachment level (in mm).

Assuming that the efficacy of the therapy can be related to the disease advancement, the clinical parameters were assessed separately for the pocket depths <5 mm and ≥5 mm.

The results were subjected to statistical analysis using the SPSS 8.0 PL packet. The Wilcoxon pairs test was used to compare changes in the parameters at time intervals in the respective groups. Differences with $p \leq 0.05$ were considered statistically significant.

Results

In all the groups, PI was significantly reduced after 3 months as compared to the baseline. The most substantial differences in this parameter were noted in the Corsodyl group, being 1.6 for pocket depths <5 mm and 1.7 for those ≥5 mm. SBI and GI were also significantly reduced after treatment. The greatest difference in these parameters was observed in group III, where apart from Corsodyl gel surgical dressing was applied, and in the control group. Pocket depths after treatment were markedly reduced in groups I, III and IV. In group II, this parameter decreased significantly for the pockets ≥5 mm, but not for <5 mm. No significant changes were observed after three months in the attachment level in group I after Corsodyl fluid and in group II for the pockets <5 mm. However, this parameter changed markedly for the pockets deeper or equal to 5 mm in patients treated with gel. In groups III and IV, the attachment level was significaltly decreased on examination 3. The differences were more pronounced in group III and depended on the pocket depth (0.9 and 2, respectively). It should be emphasized that for most of the clinical parameters examined in the study, major differences between baseline and the follow-up examinations referred to the pockets deeper or equal to 5 mm. Numerical data (mean, standard deviation and p value) have been presented in *Tab. 1-4*.

Discussion

In the current study, we achieved a significant improvement in the clinical parameters in all the groups. PI was most reduced in group I, where the mean difference between the baseline and examination 3 (after three months) was 1.65, in the remaining groups being 1, 1.2 and 1.55, respectively. Other authors have shown a similar degree of PI reduction. Mouth rinsing with 0.2% chlorhexidine solution can reduce this parameter by 1.27, gel by 1, while scaling by 1.2 [13]. Chlorhexidine used for mouth rinsing by subjects who do not perform any other hygienic procedures causes a two-fold reduction in plaque accumulation as compared to the placebo-using subjects [14].

According to Lang et al. [5], the use of chlorhexidine solution decreases GI by 18%. In our study, the GI reduction was more pronounced, being 44%-57% on average. Our results well correspond to those reported by Vinholis et al. [13], who showed a reduction in GI by 0.77 after mouth rinsing, by 0.5 after gel

Table 2. **Assessment of clinical parameters after application of Corsodyl gel with regard to periodontal pocket depth**

Parameter	Group I (<5 mm)			Group II (≥5 mm)		
	Examination			Examination		
	I	II	III	I	II	III
PI	1.7±0.95	0.8±0.79* p=0.007	0.7±0.67** p=0.01	2.0±0.53	1.0±0.53* p=0.0000	1.0±0.41** p=0.0000
SBI	2.2±1.23	1.5±0.97* p=0.01	0.8±0.79** p=0.007	2.8±0.90	1.9±0.58* p=0.0000	1.0±0.56** p=0.0000
GI	1.6±0.97	1.1±0.74* p=0.04	0.6±0.52** p=0.01	1.9±0.58	1.5±0.63* p=0.0015	0.9±0.31** p=0.0000
Clinical attachment level	3.4±1.11	3.5±1.26	3.4±1.33	7.2±1.72	6.3±1.83* p=0.0000	6.0±1.81** p=0.0000
Pocket depth	3.4±0.57	3.1±0.72* p=0.01	3.1±0.66	5.9±0.69	5.0±0.82* p=0.0000	4.6±0.81** p=0.0000

* – statistically significant difference between examination I and II; ** – statistically significant difference between examination I and III

Table 3. **Assessment of clinical parameters after application of Corsodyl gel + surgical dressing with regard to periodontal pocket depth**

Parameter	Group I (<5 mm)			Group II (≥5 mm)		
	Examination			Examination		
	I	II	III	I	II	III
PI	2.1±0.99	1.5±0.97* p=0.04	0.9±0.99** p=0.007	1.9±0.91	1.3±0.84* p=0.0000	0.7±0.74** p=0.0000
SBI	3.2±1.40	1.8±1.40* p=0.005	1.1±0.99** p=0.005	2.9±1.22	1.5±1.15* p=0.0000	1.0±0.87** p=0.0000
GI	2.2±0.92	1.5±0.85* p=0.01	1.1±0.99** p=0.007	2.0±0.88	1.4±0.76* p=0.0000	0.8±0.73** p=0.0000
Clinical attachment level	4.1±1.57	3.7±1.31* p=0.03	3.6±1.28** p=0.01	6.9±1.81	6.0±2.05* p=0.0000	5.0±1.72** p=0.0000
Pocket depth	3.8±0.58	3.3±0.47* p=0.006	2.9±0.48** p=0.005	6.1±1.59	5.1±1.79* p=0.0000	4.1±1.42** p=0.0000

* – statistically significant difference between examination I and II; ** – statistically significant difference between examination I and III

Table 4. **Assessment of clinical parameters in the control group with regard to periodontal pocket dept**

Parameter	Group I (<5 mm)			Group II (≥5 mm)		
	Examination			Examination		
	I	II	III	I	II	III
PI	2.4±0.52	1.4±0.52* p=0.007	0.9±0.74** p=0.005	2.4±0.50	1.3±0.47* p=0.0000	0.8±0.72** p=0.0000
SBI	3.6±0.97	2.2±0.79* p=0.005	1.5±0.85** p=0.005	3.6±1.02	2.2±0.78* p=0.0000	1.5±0.86** p=0.0000
GI	2.5±0.53	2.0±0.67* p=0.04	1.4±0.52** p=0.005	2.5±0.51	1.9±0.60* p=0.0001	1.4±0.50** p=0.0000
Clinical attachment level	4.4±1.48	4.0±1.41* p=0.01	3.7±1.17** p=0.008	6.9±0.17	6.4±1.18* p=0.0000	6.5±1.30** p=0.0002
Pocket depth	4.0±0.75	3.5±0.72* p=0.005	3.5±0.80** p=0.005	6.2±0.97	5.7±0.95* p=0.0000	5.8±1.00** p=0.0002

* – statistically significant difference between examination I and II; ** – statistically significant difference between examination I and III

application and by 0.9 after scaling. We found this parameter to change by 0.95 and 1.05 on average in the fluid and gel groups, and by 1.1 in the scaling group. We observed the most pronounced drop in GI (mean 1.15) in the group where Corsodyl gel application was followed by the use of surgical dressing onto the marginal gingiva to prevent the gel leaking from the pockets. This group had also markedly reduced SBI. As demonstrated in human and animal studies, at the analogous PI levels, bleeding was considerably reduced in chlorhexidine-treated subjects as compared to the control without pharmacotherapy [5,15-17].

Moreover, mouth rinsing with chlorhexidine solution allows pocket depth reduction by approximately 0.4-0.5 mm, [17] which

is consistent with our own data. Other reports provide evidence that both after scaling and application of fluid or gel with chlorhexidine the pockets diminish their depth by approximately 3.1-3.5 mm, which indicates that chlorhexidine and scaling have similar effects on the attachment level. Mouth rinsing with chlorhexidine solution and scaling caused a 3 mm decrease in the attachment level, while application of gel with chlorhexidine resulted in a 3.4 mm reduction [13]. In our study, the attachment level was most markedly changed in the surgical dressing group. Both pocket depth reduction and attachment level gain may depend on the baseline values of the above parameters [16].

Concluding our results, the most pronounced differences between the baseline and follow-up examinations occurred in the Corsodyl gel + surgical dressing group and in the scaling group. Considerable improvement in the parameters in subjects who did not receive pharmacological treatment is not surprising and confirms that in many cases scaling and root planing are so effective in the treatment of periodontitis that pharmacology is unnecessary. Large differences in the values of the study parameters between the preliminary and the follow-up examinations could be the result of the lowest baseline values of these parameters in the control group. Patients were randomly selected to the respective groups and thus the baseline values were accidental as well. Undoubtedly, the use of surgical dressing influenced the action of gel with chlorhexidine on periodontium status in patients with P. Our results would thus confirm the thesis that the efficacy of the treatment of periodontitis by means of gel application to the pockets depends on both the possibility of achieving biologically significant concentration of the drug and on the adequately long drug maintenance in the periodontal pocket [18].

According to some authors, the mode of drug administration can exert an effect not only on the clinical parameters but also on subjective sensations patients experience during treatment. Chlorhexidine has many side effects, especially when administered as mouthwash, such as brown discolouration of teeth, fillings and oral soft tissues, mainly the tongue. Patients complain of bitter and difficult to hide taste of chlorhexidine-based preparations and have taste disorders [19]. However, these unpleasant sensations are compensated by beneficial effects of chlorhexidine therapy. Additionally, chlorhexidine compounds attenuate the adhesion of *Porphyromonas gingivalis* to epithelial cells and inhibit the activity of metaloproteinases 2, 8 and 9, which is another antibacterial mechanism [20,21].

Therefore, this compound should be more frequently used as a drug adjunct to classic periodontal therapy, especially in the forms allowing its direct application to the periodontal pockets.

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Evaluation of mCD14 expression on monocytes and the blood level of sCD14 in patients with generalized aggressive periodontitis

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Abstract

Purpose: **Lipopolysaccharides (LPS), a major component of the cell membrane of gram-negative bacteria, are the main stimulants of the host immune response, initiating inflammatory changes and responsible for periodontal tissue destruction. The mCD14, which is found primarily on monocytes and macrophages, is the key membranous receptor involved in LPS binding. CD14 is also present in the serum as a soluble form (sCD14) released due to shedding from monocytes.**

The aim of the study was to assess CD14 expression on peripheral blood monocytes in patients with generalized aggressive periodontitis (GAP). The level of sCD14 was also determined in the serum of GAP patients.

Material and methods: **The study group consisted of 16 patients with generalized aggressive periodontitis, the control group had 13 systemically and periodontally healthy subjects. The expression of mCD14 was determined by flow cytometry and expressed as mean intensity of fluorescence (MIF). Serum sCD14 level was examined with ELISA method.**

Results: **The expressions of mCD14 on monocytes in GAP patients and control subjects were comparable. No statistically significant differences were noted in the mean serum sCD14 level between GAP and control subjects.**

Conclusions: **As periodontitis is a local disorder affecting a small fragment of the oral cavity it seems likely that chronic bacterial infection existing there is not reflected in the peripheral parameters.**

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Introduction

Aggressive periodontitis (AP) is a particular type of periodontal tissue inflammation, characterized by rapid destruction of tooth-supporting tissues and leading to preterm dentition loss in young people. It is believed that this course of the disease can be caused by certain individual susceptibility and genetic phenotype predisposing to severe periodontal tissue destruction [1,2]. Periodontal destruction occurs due to spread of inflammation which originally acted as host defensive mechanism and was not suppressed at an appropriate moment [3].

Anaerobic gram-negative bacteria are the major etiological factor leading to periodontitis. Lipopolysaccharides (LPS), which are present in the cellular walls of the bacteria, act as the most important stimulants of the host immune response which initiates inflammatory changes and periodontal tissue destruction [4]. The mCD14 found primarily on monocytes and macrophages and more seldom on activated neutrophilic granulocytes (PMN) is the key membranous receptor responsible for LPS binding [5]. 90% of monocytes axhibit strong CD14 staining. These $CD14^{++}$ cells are CD16-negative simultanously. Another population of monocytes are double-positive $CD14^{+}CD16^{+}$ cells which show a low level of CD14 expression and strong CD16 expression [6]. CD14 is a 55 kDa glycoprotein (glycosol-phosphatidyl-inositol). LPS is bound to CD14 by LPS-binding protein (LBP) present in the blood serum [7]. LPS, TNFα and IFNγ cause an increase in CD14 expression on human myeloid cells in vitro, while IL-4 decreases it [8]. CD14 is also found in the serum as a soluble form (sCD14) that appears due to proteolytic cleavage and phospholipase D induced shedding from monocytes [8]. Binding of mCD14 to LPS induces a number of cell activities, including production of proinflammatory cytokines (IL-1, IL-6, IL-8, IL-12, TNFα) and anti-inflammatory cytokines (IL-10, TGFβ), as well as NO or oxygen radicals [9]. Most of these compounds affect alveolar bone

Table 1. **Morphological parameters of blood in AP and C subjects (mean ± standard deviation)**

	AP	C	p
WBC	6.1±1.3	5.35±1.2	p=0.74
MO%	8.3±2.9	6.3±2.9	p=0.12
MOan	0.5±0.2	0.5±0.3	p=0.54

WBC – white blood cells, MO% – % of monocytes; MOan – absolute number of monocytes

Table 2. **mCD14 on peripheral blood monocytes and sCD14 level in sera of GAP and C groups (mean ± standard deviation, minimum and maximum values)**

Parameter	GAP			C		
	mean	minimum	maximum	mean	minimum	maximum
mCD14	25.08±5.26	14.3	31.8	27.02±3.03	22.5	32.9
sCD14	1759±277	1294	2328	1796±165	1540	2006

resorption [4]. The role of sCD14 remains not fully elucidated. This molecule blocks binding of LPS by mCD14 and thus inhibits cell activation [8].

Immune system deficiencies towards the periodontopathogens have been reported in AP patients [1,3,10]. Therefore the question arises wheter abnormalities of mCD14 expressoin on blood monocytes and sCD14 serum concentration exists in AP patients and if these abnormalities can be involved in the etiology of aggressive periodontitis.

That's why the aim of the present study was to assess the mCD14 expression on peripheral blood monocytes and serum sCD14 level in patients with aggressive periodontitis.

Material and methods

16 generally healthy, no smoking GAP patients, aged 23--45 years (mean 37.5), 10 women and 6 men, were involved in the study. The control group (C) consisted of 13 periodontally healthy subjects, aged 25-47 years (mean 38.5), 8 women and 5 men. The subjects of control group were non-smokers who had not taken any drugs (including antibiotics) for the previous 6 months. The diagnosis was based on clinical and radiographic examination according to criteria of classification recomended by American Academy of Periodontology [11]. The control subjects had no history of periodontal disease (no bleeding on probing, probing pocket depth <3 mm, no attachment loss, lack of bone loss on radiographs). In GAP patients moderate or severe destruction of periodontal tissue were assesed. These patients had undergone rutine periodontal therapy (scaling and root planing, farmacological and surgical therapy) at least two years ago. All the study subjects gave their written consent for the collection of the research material. The study was approved by the Bioethics Committee of the Medical University of Białystok.

Blood for analysis was collected in the morning hours from the ulnar vein to a test-tube containing EDTA-K_3 as anticoagulant. Morphological parameters with full differentiation of peripheral blood leukocytes were assessed using a hematological analyzer Coulter MAXM (Coulter, USA). Results were expressed as absolute values (G/l) and percentage values (%). Monoclonal anti-CD14 antibodies marked with fluorescein isotiocianine (FITC) (DakoCytomation, Denmark) were used for study. Standard techniques of direct fluorescein labeling of whole blood leukocytes were used to assess antigen expression. 10μl of the monoclonal antibody was added to 100 μl blood and after 15-min. incubation at room temperature, with no access to light, the sample was processed by means of rapid lysis using EPICS IMMUNOLOGY WORK STATION with ImmunoPrep Reagent System. Following careful mixing, the sample was examined in a flow cytometer EPICS XL (Coulter) equipped with argon laser 488 nm. Each time, the apparatus was calibrated with DNA Check. Results were expressed as MIF – mean intensity of fluorescence, indirectly reflecting density of the molecule examined on cells. All the above tests were made from fresh samples of blood.

The ELISA method was applied to assess serum concentrations of sCD14, using a Quatikine Human sCD14 Immunoassay Kit (R&D, USA). Serum samples for ELISA analysis were stored at -80°C. Results were expressed in ng/ml.

Data were subjected to statistical analysis using Mann-Whitney test with SPSS 8.0 PL program. Statistically significant differences were considered at $p<0.05$. The Pearson test was applied to estimate the correlation.

Results

Mean morphological parameters (leukocytosis, % and absolute monocyte count) were similar in both groups (*Tab.* 1). Mean fluorescence value of mCD14 on monocytes in AP patients was comparable to control (p=0.28). No statistically significant differences were found in the mean serum sCD14 levels between GAP patients and control subjects (p=0.63). In both groups considerable individual differences in the parameters examined were observed. Numerical data referring to GAP and control groups have been presented in *Tab. 2*. There were no correlation found between expression of mCD14 and serum levels of sCD14 in GAP and control groups.

Discussion

The expression of mCD14 on monocytes and serum sCD14 level have been examined by many authors in various generalized inflammatory diseases. A significant increase has been found in these parameters in e.g. HIV-positive subjects, patients with acute vasculitis, systemic lupus erythematosus (SLE), sepsis, malaria and patients after hemodialysis [8,12-15].

In our study on the expression of mCD14 on monocytes no evident differences were noted between GAP and control subjects. At the same time, in both groups we found quite substantial differences in the parameters examined between the respective individuals. Our results seem to correspond with those of Buduneli et al. [16], who examined patients with various forms of periodontitis (P). These authors revealed no differences in the expression of mCD14 on monocytes between AP and control subjects. However, Shapira et al. [17] found a decrease in mCD14 expression on monocytes in AP patients.

In our study, also serum sCD14 level in GAP patients was similar to its level in healthy subjects. Hayashi et al. [18], however, found an increase in serum sCD14 level in patients with various forms of periodontitis. They noted that dental plaque LPS penetrating the gingiva had the most significant effect on sCD14 release. An increase in the plaque mass enhances the inflammatory reaction in periodontal tissue. With the progression of the inflammatory symptoms, bacterial products may get to the blood, thus stimulating sCD14 secretion by monocytes in P patients [18,19].

Also expression of this molecule after periodontal treatment was evaluated. The sCD14 concentration in patients after treatment was significantly decreased, reaching the level of healthy subjects [18]. This can partly justify our study outcome. Our patients had been previously treated (at least two years before the study) according to standard periodontal hygienic and surgical procedures, which brought considerable clinical improvement. In the study period, the patients had only slightly intensified symptoms of inflammation: gum swelling and bleeding from periodontal pockets during probing. It can be thus assumed that in AP patients serum sCD14 level can be close to the norm in the chronic phase of inflammation, though altered in the acute phase.

Other studies point at the likely relationship of CD14 with the pathomechanism of P. It has been found that the sCD14-LPS complex affects the activation of cells which originally showed no mCD14 expression, namely endothelial cells and fibroblasts of gingival tissue. Endothelial cells affected by this complex release inflammation mediators, whereas fibroblasts exhibit higher expression of intercellular adhesion molecule 1. On the other hand, sCD14 can play the role of carrier protein and transport LPS to high density lipoprotein (HDL) [8,20,21].

Attempts have been also undertaken to assess CD14 in periodontal tissues and in gingival crevicular fluid (GCF). McNamara et al. [22] found no differences in the levels of sCD14 in GCF between the CD14 monocyte receptor deficient patients and healthy subjects. Other authors, even though they observed no statistical differences between the levels of this molecule in GCF in P patients and healthy subjects, found certain correlation with clinical state. Namely, low sCD14 level in GCF was observed in patients with deeper periodontal pockets (>5 mm) and with their greater number [23]. Hayashi et al. [20] showed lack of gingival fibroblast staining by mCD14 antibodies. The authors cited above suggest that sCD14 play a basic role in the response to LPS present in periodontal pockets, and thus in bacteria-induced periodontal damage. Another authors have studied the in vivo espresssion profile and levels of mCD14 in healty and diseased gingival tissues. Clinically healty tissues showed greater levels of mCD14 than periodontal pocket tissues what may suggest that mCD14 is associated with favorable host responses to bacteria and may play role in maintaining periodontal homeostasis [24].

In conclusion basing on literature data it can be assumed that mCD14 and sCD14 play role in pathogenesis of aggressive periodontitis. However the relationship of these molecules with pathogenesis of periodontits is still unclear and needs further investigatons. According to our own results it can be taken into consideration that periodontal inflammation is a local disorder affecting a small fragment of the oral cavity and it seems likely that the chronic bacterial infection taking place there is not reflected in the peripheral parameters.

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The assessment of periodontium in patients with uncontrolled diabetes

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Abstract

Purpose: Uncontrolled diabetes leads to disturbances of carbohydrate, protein, and lipid balance as well as morphological changes in many organs. It can be assumed that the changes can also regard the masticatory organ and thus, periodontal tissues.

The aim of the study was the assessment of periodontium in patients with uncontrolled diabetes (HbA_{1C} >7%) and the comparison of the results with data obtained in the group of healthy individuals – depending on sex.

Material and methods: The study was carried out in the group of 275 subjects: 155 hospitalized patients with uncontrolled diabetes (the examined group) and 120 healthy individuals comprising the control group. Russell's index was used for the evaluation of the periodontal condition.

Results: The mean level of glycated hemoglobin HbA_{1C} in patients was 9.43% in women and 9.57% in men. The mean value of Russell's index was 2.14 in the examined group and 0.99 – in the controls. The difference was statistically significant ($p<0.001$).

Discussion: Although other authors' results are ambiguous and controversial, the theory that there is the connection between uncontrolled diabetes and periodontitis and the consequences of the coexistence of these diseases are very serious, is still maintained.

Conclusions: Uncontrolled diabetes was the crucial cause of periodontal changes and, to a large extent, influenced the function of the masticatory organ in patients.

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Introduction

Diabetes mellitus, specifically uncontrolled diabetes, is the disease most frequently mentioned as the systemic metabolic disease affecting the condition of the masticatory organ, and thus periodontium [1].

Diabetes mellitus is considered to be a social disease. According to the epidemiological data, there are 3% to 6% of the society suffering from the disease. Diabetes can markedly influence general indices of morbidity and mortality and lead to life shortening by about 30% [2,3].

The disease can be defined as the systemic disorder characterized by hyperglycemia, which is a result of insulin secretion or action defects [3].

The following types of diabetes mellitus are distinguished: type 1 diabetes (autoimmunologically conditioned or idiopathic), type 2 diabetes, other specific forms of diabetes (occurring, e.g. in the course of infectious diseases, chronic pancreatitis, endocrinopathy, genetic diseases, connected with drug taking), and diabetes in pregnancy [3-5].

The ailment is characterized by the risk of complication occurrence, both acute and chronic [4,6]. All chronic diabetes complications are probably due to vascular disorders, which can be divided into non-specific macroangiopathy and specific microangiopathy. It seems that the influence of vascular lesions in the course of diabetes mellitus on periodontal tissues is unquestionable: capillary and precapillary angiostenosis hinders the transport of nutritional components. Oxygen diffusion and waste products elimination impairment leads to physiological imbalance and increases periodontal sensitivity to injury [7]. According to Matthews [8], chronic diabetes complications are connected with persistent hyperglycemia, which results in the formation of end products of advanced glycation (AGEs). They sensitize endothelial cells and monocytes to stimuli inducing inflammatory mediators. As plasma and tissue AGEs accumu-

Table 1. **Mean glycated hemoglobin HBA_{1C} in examined group (depending on the sex)**

HBA_{1C}	Subgroup I			Subgroup II			Examined group (total)		
	M	F	Total	M	F	total	M	F	total
Number	34	36	70	46	39	85	80	75	155
Standard deviation	1.15	1.58	1.38	1.86	1.3	1.63	1.49	1.44	1.52
Medium value	9.7	9.8	9.8	9.7	9.6	9.6	9.7	9.6	9.6
Mean value	**9.43**	**9.51**	**9.47**	**9.68**	**9.36**	**9.53**	**9.57**	**9.43**	**9.5**

Table 2. **Mean Russell's index value in examined and control groups**

Russell's index	Subgroup I			Subgroup II			Control group			Examined group (total)		
	M	F	total	M	F	total	M	F	total	M	F	total
Number	24	28	52	30	29	59	51	54	105	54	57	111
Standard deviation	1.01	1.27	1.18	0.87	0.94	1.05	0.85	0.78	0.81	1.18	1.11	1.15
Medium value	1.57	1.8	1.72	3.01	2.02	2.5	0.7	0.8	0.8	2.55	2.0	2.02
Mean value	1.5	2.05	1.79	2.97	1.89	2.44	0.96	1.02	0.99	2.32	1.97	2.14
Statistical analysis	0.095			0.001			0.708			0.108		
	0.001						0.001					
	0.018											
				0.001								
	0.595									0.001		
	0.001											
				0.001								
				0.003						0.001		
				0.001								
							0.001					

lation occurs due to uncontrolled diabetes, it can be assumed that periodontal tissues with large amount of AGEs content are characterized by higher vascular permeability, elevated collagen fiber atrophy, and reveal accelerated damage of non-mineralized connective tissues and bones.

The aim of the study was the assessment of periodontium in patients with uncontrolled diabetes mellitus (HbA_{1C} >7%) and the comparison of the results with those obtained in the group of generally healthy individuals – depending on sex.

Material and methods

The study was performed in the group of 275 patients: 155 hospitalized patients with type 1 (subgroup I) or type 2 uncontrolled diabetes (subgroup II) – the examined group, and the control group of 10 healthy subjects. The examination was carried out at a dental surgery, in artificial light, with the use of a dental set and a calibrated periodontological probe. The results were included into prepared examination charts. Russell's index was used to evaluate periodontal condition.

The results were transferred into electronic data base, which enabled the statistical analysis. The hypotheses of the study were verified with t-Student test for two means and "u" test for two frequencies. Differences at $p<0.05$ were considered statistically significant.

Results

The mean glycated hemoglobin HbA_{1C} level in patients of the examined group was 9.43% in women and 9.57% in men (*Tab. 1*). The subgroup 1 revealed the mean value of 9.47% and was slightly higher in women – 9.51% than in men – 9.43% while in the subgroup II it was 9.53% (9.36% in women and 9.68% in men, respectively).

The mean value of Russell's index (*Tab. 2*) was 2.14 in the whole group while in the control group it was 0.99. The difference was statistically significant ($p<0.001$). The Russell's index in the subgroup I was on average 1.79 and in the subgroup II it was markedly higher and constituted 2.44. As far as the subgroups are concerned, the difference was statistically significant ($p<0.003$). It points to more advanced periodontal lesions in patients with uncontrolled diabetes of both types.

Men in the whole examined group revealed a slightly higher Russell's index than women (2.32 and 1.97, respectively) whereas the difference between mean values of the index (men – 2.97 and women – 1.89) in patients of subgroup II was statistically significant ($p<0.001$) in favor of women.

Discussion

There are many reports in the literature concerning the influence of uncontrolled diabetes on periodontal condition. However, the spectrum of examinations was very narrow and the

choice of indices – diverse in character. Other authors' results are ambiguous and controversial. According to Soskolne et al. [9], the dynamics of pathological processes in periodontium in the course of diabetes mellitus depends on numerous factors, like compensating the disease and the presence of vascular complications. Firatli [10] claimed that vascular microangiopathies of gingivae lead to disturbances in oxygen distribution and waste products elimination as well as leukocyte migration defect. Thus, it can be assumed that these factors decrease the ability of periodontal tissues to repair and regenerate in patients with diabetes mellitus. Grossi et al. [11], who studied microbiological indices of periodontal diseases in 1426 patients, showed that the risk of periodontitis occurrence in patients with diabetes was 2-3 times higher than in healthy subjects. Löe [12] observed a sixfold increase in the rate of periodontal diseases in diabetics than in healthy. The studies of Hallman and Mealey [13] and, independently Szymańska and Fetkowska [14], revealed more frequent occurrence of oral mucositis in patients as compared to the healthy. A marked elevation of periodontal diseases was observed in patients with type 1 diabetes than in those with type 2 [15], despite the fact that Saito et al. [16] claimed that type 2 diabetes is the higher risk factor in these diseases.

Thus, other authors' results are in accordance with ours. However, some scientists do not seem to notice the differences between periodontal tissue condition in patients and healthy subjects. Hayden and Buckley from Ireland [17], and independently Vechis-Bon from France [18], calculating periodontal indices and assessing the depths of gingival pockets and glycated hemoglobin level in patients with diabetes did not show any significant correlation between diabetes and periodontal diseases.

Despite those controversial results, the theory of the connection between the two diseases and the consequences of their coexistence are very serious, is maintained [19,20].

Conclusions

Uncontrolled diabetes constituted the significant cause of periodontal tissue changes and the marked loss of the masticatory organ functioning in patients. Thus, the effect of this ailment on periodontal tissues, and the masticatory organ itself, comprises a serious problem and should become the scope of intensified preventive and therapeutic activities of diabetologists and dentists.

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Multidisciplinary treatment of patients after a surgery due to cancers in the facial area: a clinical reports

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Abstract

Prosthetic rehabilitation of patients after surgical removal of carcinoma in the facial skeleton is one of the most difficult problems in therapy of the stomatognathic system, due to increasing incidence of head and neck carcinoma. Significant deformations of tissues, development of dysfunctions of the stomatognathic system with concurrent biological unbalance of the oral cavity enviroment are frequently a consequence of the treatment. Cicatricial scars, contraction of the oral crevice and limitation of mobility of the tongue are noted in numerous cases. Deformations of the facial area of the skull and of structures of the temporo-mandibular joint are also the reasons of occlusion and articulation disturbances. Two cases of surgery due to carcinoma in the facial skeleton that have required combined and stepwise multispecialistic treatment performed at Department of Prosthetic Dentistry and Department of Periodontology and Oral Disease, Dental Institute, Medical University of Warsaw are presented. The therapy has involved treatment of periodontitis and applying appropriate construction of prostheses that would relieve periodontium and splinting teeth.

Key words: head and neck neoplasms, radiotherapy, periodontitis, multidisciplinary treatment.

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Introduction

Surgical procedures supplemented with radiotherapy or chemotherapy are one of fundamental significance in the treatment of tumours of the maxillofacial region. The aim of an adjuvant therapy is to destroy neoplastic cells; but it is burdened with numerous side effects [1-5]. Consequently, a syndrome of clinical symptoms characterised by xerostomia, inflammatory changes of the oral mucosa and lowering of salivary pH may develop. This is often accompanied by increased caries and deposition of dental plaque, sometimes involving all tooth surfaces. Pathological attrition is also observed as a result of rapidly progressing demineralization of the hard dental tissues. There is a high incidence of caries and pathology in the region of the periodontal tissues and oral mucosa, particularly in patients subjected to irradiation. The increased incidence of caries in this group of patients is caused by the direct action of ionising radiation on the hard dental tissues and disturbances of the oral-cavity biocoenosis, reduced saliva production, and a fall in its pH. Caries most commonly appears in the cervical region of the teeth in irradiated patients. In the majority of cases, it is encircling caries involving almost all of the teeth, therefore preventive measures are necessary in addition to treatment of the caries itself. Serious defects in speech and impaired swallowing can also occur. Major changes in facial appearance in postoperative patients are often the cause of depression [6-10].

Aim of study

All of the described changes and disturbances pose serious difficulties in reconstruction of the stomatognathic system. The aim of our paper was to describe selected cases that required combined and staged multidisciplinary treatment carried out at the Department of Prosthetic Dentistry and the Department of Periodontology and Oral Diseases, Dental Institute, Medical University of Warsaw.

Materials and methods

In postoperative patients who had procedures because of neoplasms in the facial region, attention was paid to the symptoms described in the medical history (dryness in the oral cavity, pain, burning, disturbances of taste, difficulty in swallowing food). The number of cigarettes smoked, the presence of coexisting organic and systemic disease and medication used were taken into account.

In the clinical examination of the oral cavity, attention was paid to the condition of the oral mucous membrane, tongue, teeth, periodontal tissues, and to oral hygiene.

The mucous membrane was examined using a dental mirror. Clinical examination of the teeth was carried out using a probe and the periodontal tissues were examined using the Florida Probe package, equipped with a computer-linked constant pressure probe, and with a WHO periodontal probe.

The periodontal examination consisted of meaning pocket depth and the level of connective tissue attachment loss (in mm). The results of these examinations were subjected to analysis taking into account the maximum values for a given patient (PDmax and CALmax) and the mean value for a given patient (PDmed and CALmed). The plaque and bleeding indices according to O'Leary were evaluated (they express the percentage relationship of bleeding measurement points on probing in 6 examined sites bleeding index and number of surfaces covered with plaque in 4 examined sites, plaque index, in all sites of measurement). Tooth mobility was assessed using the tooth mobility index according to Entina. The quantity of non-stimulated mixed saliva secreted was also measured, by collection over 5 minutes into a calibrated container (the method of Edgar and O'Mullane). This examination was carried out in the morning, at least 1 hour after breakfast. The patients were recommended to have mycological examinations (direct smear of the mucosa of the postoperative defect and tongue) to ascertain what fungi were present (with an antimycogram carried out using the diffusion-disc method).

The periodontal therapy included an initial phase of periodontal treatment consisting of professional cleaning of teeth and a corrective stage in which surgical procedures were performed on the periodontium. Due to the presence of dispersed postoperative defects, significant deformation of maxillofacial tissues, and temporo-mandibular joint and articulation-occlusion disturbances, the prosthetic reconstruction required the use of special treatment methods and the construction of prostheses that restored tissue defects and tooth loss, and which splinted retained teeth.

Case reports

Case 1

An 58-year-old man, after surgery of *carcinoma maxillae lateralis dextri* at the Department of Maxillofacial Surgery, Medical University of Warsaw. Partial resection of the right maxilla together with excision of submandibular lymph nodes on the right-hand side was performed in December 2000. Next, the patient was subjected to radiotherapy at a dose of 6000 cGy on the maxillary-cribriform region and 5000 cGy on the neck. He was referred to the Department of Oral Medicine and Periodontology because of symptoms of burning and dryness in the oral cavity and associated difficulties in speaking and swallowing of food. The patient was not able to collect saliva for quantitative analysis and could speak only with frequent moistening of the oral cavity with water. The patient did not smoke and did not take any medicines. Extraoral examination showed an extensive scar on the facial skin and on the neck on his right side, dry and cracked lips and inflammation of the corners of the mouth. Intraoral examination revealed dry oral mucosa, light pink in colour, matt appearance; lack of continuity of the hard and soft tissues of the hard palate on the right side with a defect of approximate 1.5 cm diameter; dry tongue with limited mobility and numerous scars (as reported by the patient, the scars appeared after radiotherapy). Clinical examination indicated the presence of the following teeth: 11, 21, 22, 23, 24, 34, 33, 32, 42 and 43 containing numerous carious cavities (*Fig. 1a*). The mean gingival pocket depth (PDmed) was 1.65 mm, and the mean level of loss of connective tissue attachment (CALmed) was 3.7 mm. Maximum values were as follows: periodontal pockets (PDmax) 4 mm, loss of connective tissue attachment (CALmax) 7 mm. The number of teeth with loss of connective tissue attachment above 5 mm was 21%. The plaque index was 100%; the bleeding index 71%. On the basis of history taking, clinical examination and radiographs, severe chronic periodontitis was diagnosed.

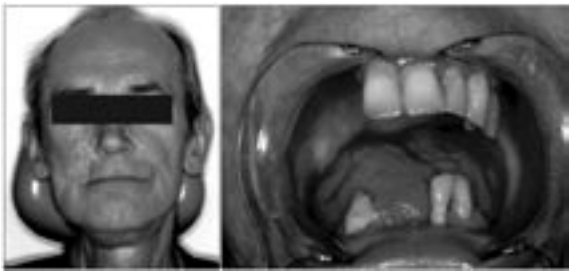
Initial phase periodontal therapy consisting of scaling and polishing using prophylaxis paste and rubber cups was carried out. Existing restorations were corrected (overhangs removed). The surfaces of all the teeth were covered with fluoride varnish – Fluor Protector. Oral hygiene instruction was given. Before commencing periodontal treatment, the patient was advised to have a mycological examination and conservative treatment of the teeth. Pharmacological therapy was used – Metronidazole (250 mg tablets [Polpharma]; 1 tablet every 8 hrs for 7 days and 0.1% pilocarpine mouthwash). A follow-up visit was scheduled after 7 days. Mycological examination was negative. Clinical examination again showed poor oral hygiene, which was indicated by a plaque index of 60%. The teeth were cleaned of deposits and oral hygiene instruction was given again. Further monthly visits were booked and the state of oral hygiene was controlled. After about two months from the beginning of treatment, the patient confirmed the appearance of saliva in the oral cavity in quantities that allowed for normal speaking. Ingestion of food without fluid was still not possible.

After 4 months, clinical examination revealed: the plaque index 30%, and bleeding index 18%. The quantity of secreted, unstimulated mixed saliva was 0.4 ml/5min.

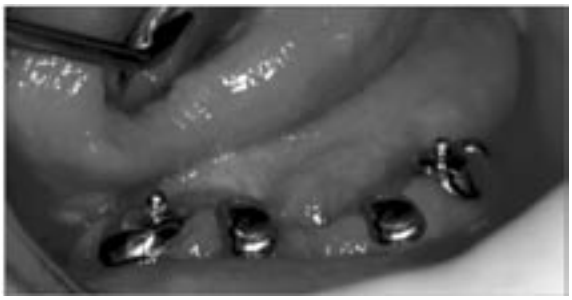
Prosthetic treatment during the first stage consisted of tissue-borne partial dentures: the maxillary with an obturator closing the oro-antral fistula, with an elastic soft lining made of Softerex (Zhermack). It allowed for better sealing of the obturator and reduction of pressure by the hard parts of the prosthesis on the poor quality mucosa in the region of the postoperative defect; the tissue-borne partial mandibular denture did not require any modification. The temporary prostheses allowed the patient to function normally at least to the greatest possible extent. The speech of the patient improved and chewing ability improved.

Figure 1. **Patient after partial resection of the maxilla with excision of submandibular lymph nodes on the right side because of carcinoma maxillae:**

a) photo of patient's face and oral cavity before treatment

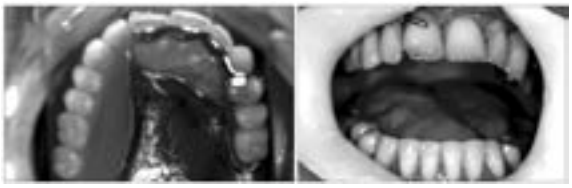


b) photo of patient's oral cavity with Rhein 83 ball retainers system on the roots of 33, 43 and roots of 34, 32, 43 with inlay posts with protective dowel caps



c) postoperative maxillary removable partial denture (RPD) and mandibular overdenture

d) photo of patient's oral cavity after treatment



These dentures also gave the patient the possibility of having conservative and periodontal treatment of the remaining teeth and they could be quickly and effectively corrected if necessary.

The second stage of prosthetic treatment was carried out after completion of conservative and periodontal treatment. In the maxilla, a removable partial denture – RPD (*Fig. 1c*, *d*) was constructed to replace the missing teeth and to splint those remaining. A complete obturator was made of acrylic and lined with Ufi-gel silicone soft lining (Voco). In the mandible, after previous endodontic treatment of 43, 42, 32, 33, 34, inlay posts with protective dowel caps on exposed root surfaces were constructed on 42, 32 and 34 and inlay posts with Rhein 83 ball retainers system on the roots of 43 and 33 (*Fig. 1b*). Next, a full mandibular overdenture was constructed (*Fig. 1c*, *d*).

Case 2

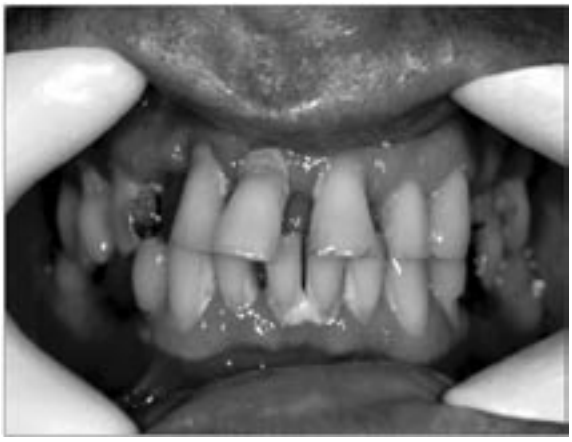
An 46-year-old man, 3 years after surgery of *carcinoma palati duri* at the Department of Maxillofacial Surgery, Medical University of Warsaw. The surgical treatment carried out in June 1998 consisted of maxillary osteotomy on the right side and resection of the hard palate. After half a year, the patient was again admitted to the department for supplementary irradiation. He received a dose of 6000 cGy on the region of the hard palate and sinuses.

The patient received prosthetic treatment immediately after the resection of the hard palate – an acrylic obturator was constructed which the patient used during the radiotherapy and during the period of tissue healing after irradiation. Twelve months after the completion of therapy, the patient turned to the Department of Prosthetic Dentistry for a further prosthetic treatment (*Fig. 2a*, *b*). During the first stage a new obturator, appropriately adapted to the new conditions in the oral cavity with a peripheral seal of plasticized acrylic Softerex was made. This distinctly improved the patient's articulation and prevented the entry of food into the maxillary sinuses. Within a short time, a partial maxillary denture with a large cup-shaped seal of plasticized acrylic Softerex was made (*Fig. 2c*). At this stage, the patient was referred to the Department of Periodontology and Oral Diseases, Medical University of Warsaw.

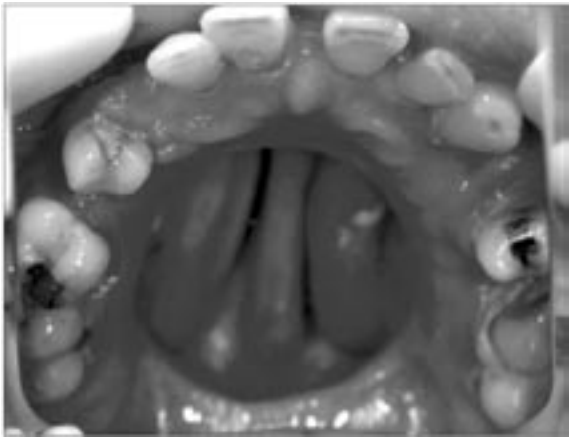
The medical history did not reveal any complaints from the patient. He was not taking any medicines and had not smoked for 2 years. Intraoral examination revealed the presence of 7 teeth in maxilla and 13 teeth in the mandibule. The mean pocket depth (PDmed) was 1.9 mm and the mean level of loss of connective tissue attachment (CALmed) was 4.2 mm. Maximum values were: periodontal pockets (PDmax) 9 mm, loss of connective tissue attachment (CALmax) 7 mm. Pockets of depth more than 5 mm made up 12% of all pockets in this given patient, whilst the number of teeth with loss of connective tissue attachment of more than 5 mm was 39%. The plaque index was 100%, and bleeding index 85%. Severe chronic periodontal inflammation was diagnosed on the basis of the medical history, clinical examination and radiographs. The quantity of unstimulated mixed saliva secreted was 1 ml/5 min. Very large quantities of deposits on the teeth were found, both supragingival and subgingival, together with a few carious cavities.

Figure 2. **Patient after osteotomy on the right side and resection of the hard palate because of carcinoma palati duri T2N0M0**

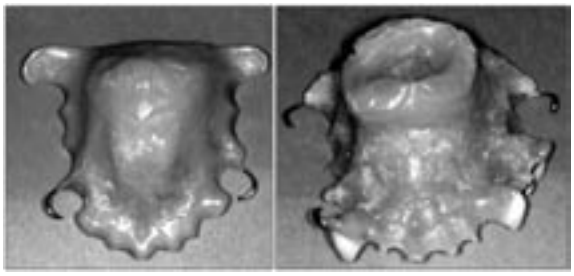
a) photo of patient's oral cavity before treatment



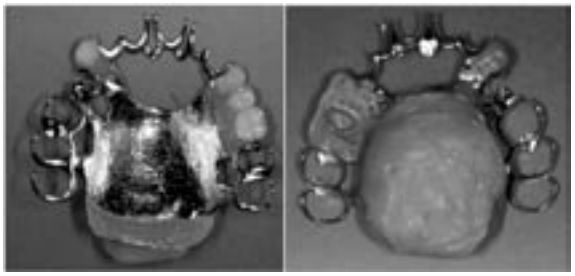
b) photo of patient's oral cavity with defect of hard palate



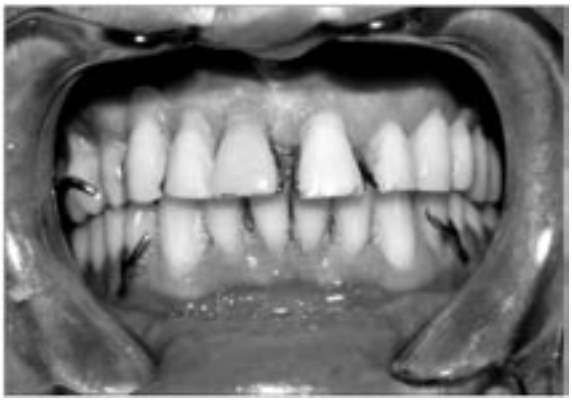
c) temporary removable partial dentures: immediate obturator plate and maxillary denture with obturator seal of plasticized acrylic Softerex

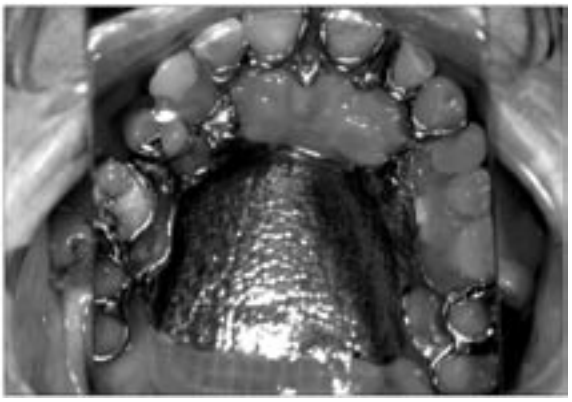


d) postoperative splinting maxillary removable partial denture (RPD) with obturator lined with Ufi-gel silicone soft lining



e) photo of patient's oral cavity with postoperative denture





Initial phase periodontal therapy was carried out, consisting of scaling with planing of root surfaces and detailed oral hygiene instruction was given. The patient was advised to have a mycological examination and conservative treatment of the teeth. Pharmacological therapy was used – Metronidazole (250 mg tablets [Polpharma]; one tablet every 8 hrs for 7 days), 0.12% chlorhexidine mouthwash (twice daily) and 0.1% pilocarpine mouthwash). The next visit was arranged 7 days later. Mycological examination was negative. Tooth deposits were again removed. On the follow-up visit after one month the plaque index was 31%, bleeding index 25%. Closed curettage was carried in the 14-25 region under local anaesthesia using Ubistesin 4%.

The second stage of prosthetic treatment was carried out after previous endodontic treatment of 11 and 25. An inlay post and core was manufactured for 11 and crown with porcelain veneer and a root inlay post with a Rhein 83 ball attachment

system in 25 was made. It was decided to construct a post and core inlay and porcelain – veneered crown for tooth 11 despite its I° mobility. This allowed for the shortening of the crown and setting it up in proper relation to the opposing teeth, and for the eradication of the traumatic bite, which could cause further mobility of 11 (radiological control 3 months after construction of prosthesis did not show any worsening of mobility of 11). Next, a splinting maxillary RPD was constructed (*Fig. 2d*). The extended arch splinting the maxillary teeth posed a certain problem of an aesthetic nature, but appropriate preparation of the approximal surfaces and incisal edges of the upper anteriors and the accurate construction of these elements by the dental technician minimalized the unfavourable aesthetic effect. The major connector of the maxillary RPD had special retention elements on its mucosal surface for the attachment of a large cup-shaped obturator whose base was constructed out of acrylic and the mucosal surface was lined with Ufi-gel elastic silicone material. The prosthesis constructed with such an obturator allowed for improvement in the function of the patient's chewing apparatus, his speech became even more distinct and less "flat", as compared with the previous prosthesis. The peripheral seal of the obturator, the stability and ruggedness of the metal frame of the prosthesis allowed the patient to return to his previous dietary habits. The missing teeth in the mandible were also replaced by a splint type of RPD (*Fig. 2e*).

Discussion

Recent studies have frequently raised the problem of changes arising in the oral cavity of patients receiving radiotherapy in the head and neck region. As a result of therapy, in the presence of unsatisfactory oral hygiene together with reduced defence mechanisms in irradiated individuals, the destruction of the tissues supporting the teeth is much more likely to occur [1,4,11]. Galler et al. described three cases of post-radiation necrosis of bone that developed in sites with active periodontal disease [12]. In the available literature, it has been shown that post-irradiation osteonecrosis most commonly occurs 3 to 6 years after irradiation and affects the mandible. Bone necrosis did not occur in patients described in our study. Dryness of the oral cavity and worsening of the general condition of the periodontal tissues did, however, develop in those patients who had coexisting poor oral hygiene.

The studies of many authors have shown the widening of the periodontal space and the disappearance of alveolar compact bone on radiographs taken at short intervals of time in patients subjected to irradiation. Fujita et al. observed widening of the periodontal space within 6 months after radiotherapy [2]. They did not, however, find destruction of periodontal tissue during the period of 3 months from commencement of irradiation. The poor condition of periodontal tissues in patients treated at the Department of Oral Medicine and Periodontology was not necessarily caused by radiotherapy alone. The coexisting poor oral hygiene (the plaque index in one of the patients was 100%) undoubtedly had an effect on the pathology of the periodontal tissues.

Professional tooth cleaning led to improvement of the periodontal tissues, as manifested by the reduction of periodontal pocket depth, rebuilding of connective tissue attachment and stabilisation of the teeth. The effects of scaling and root planing together with plaque control on the reduction of the above-mentioned parameters have been confirmed by others. Among others, Garrett et al. showed reduction in pocket depth, and reconstruction of connective tissue attachment in patients with periodontal disease. After scaling and root planing in the group examined, a reduction of pocket depth of 0.9-1.3 mm was obtained after 9 months and the value for loss of connective tissue attachment was reduced by 0.7-0.9 mm [13]

The reduction of salivary secretion observed in our patients, giving rise to a feeling of dryness in the oral cavity was undoubtedly connected with the radiotherapy. This is confirmed by the reports of other researchers [1,3]. Dreizen in his study showed that 42 patients with neoplasms in the region of the oral cavity who were irradiated with doses in the region of 200 rad daily, 5 days per week, had a reduction of 57% in the mean value of salivary flow under the influence of stimulation by chewing during the first week of therapy, of 76% six weeks postoperatively and of 95% three years after radiotherapy. Some of these patients experienced a subjective reduction of dryness in the oral cavity one month after radiotherapy, but measurement of salivary flow did not show any increase in its secretion [1]. On the basis of 60 patients after radiotherapy in the region of the head and neck, Karlsson found that reduced salivary secretion should be classified as sialopenia (salivary secretion of 0.1-1.0 ml/15 min.) or as xerostomia (0.0-0.9 ml/min.). Differences in secretion of saliva after radiotherapy depend on the site of irradiation in the region of the large salivary glands (parotid, sublingual, submandibular), and to a lesser extent on the dose of irradiation. Studies have shown that the presence of salivary glands in the region of the irradiated field is the most important factor that makes it difficult to maintain their functioning. Therefore, all preventive procedures aimed at protecting the salivary glands should be planned with the oncologist before radiotherapy is begun [3].

Prosthetic reconstruction of patients after surgery for neoplasms in the maxillofacial region poses a great challenge for the prosthetist not only because of the obvious technical problems but also because of the need for follow-up treatment. Interdisciplinary treatment and cooperation between doctors of various dental and medical specialties allow for the effectiveness of treatment and improve the general condition of the patients. Rusiniak, Ciechowicz et al. emphasise the significance of individualised treatment planning of construction design for prostheses, allowing for maximal utilisation of residual teeth for retention and stability of postoperative prostheses and also for the protection of the periodontium of these teeth and the poor-quality denture foundation [7,8,14,15]. Dreher demonstrates the significance of regular follow-up examinations, dental, radiological, mycological and adjustment of prostheses. He also demonstrates the importance of using appropriate soft reline materials with the aim of protecting the underlying mucosa and bone [8,16,17]. Our observations over many years, supported by the opinion of many investigations [6,14-16], indicate that in patients who attend follow-up visits regularly and who allow for the adjustment of prostheses and the evaluation of the underlying tissues, and for on-going prosthetic treatment (change

of prostheses when they do not fulfil their function), positive results of rehabilitation of the stomatognathic system more frequently are obtained.

Conclusions

1. Prosthetic rehabilitation of patients after extensive procedures for the removal of neoplasms in the maxillofacial region should, aside from the construction of prostheses, should include multidisciplinary treatment and prevention for protecting the poor-quality denture foundation.

2. Treatment procedures, conservative and periodontal, should be aimed to preservation of the greatest number of patient's own teeth to improve the retention of prostheses and to achieve a satisfactory function.

3. Multidisciplinary treatment of patients after surgery in the maxillofacial region, especially after radiotheraphy, allows for better prosthetic reconstruction and maintenance of good long-term results for these patients.

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The choice of conditions for cathepsin D activity determination in human saliva

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Abstract

The aim of the study was the demonstration and choice of conditions for the determination of cathepsin D activity in human mixed saliva. The 6% solution of hemoglobin, denatured with hydrochloric acid, was used as the substrate. The ratio of saliva volume to hemoglobin was 4:1 w/v. The reaction was interrupted by adding 10% trichloroacetic acid, after 6 hours of incubation at 37°C. The increase in degradation products was determined with the use of Folina and Ciocalteau method with copper modification.

Key words: human saliva, cathepsin D, method of determination, inhibitor from Vicia sativa L.

Introduction

Cathepsin D (EC 3.4.23.5) is an aspartyl endopeptidase [10]. It splits the bindings of carboxyl groups of hydrophobic aminoacid residues. It is a lysosomal enzyme acting in the acidic environment. It also occurs in small amounts in the systemic fluids, secretions, and excrements [8].

Cathepsin D activity is usually determined using protein substrates: hemoglobin, casein, and albumin [9].

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Material and methods

Dithiothreitol (Serva, Germany); hemoglobin (Difco Laboratories, USA); cathepsin D inhibitor in common vetch seed coats [4]; 2,4,6-trinitrobenzensulfonic acid and ninhydrin reagent (Sigma, USA); Folin-Ciocalteau reagent (Merck Germany). Other reagents POCh, Gliwice.

The mixed saliva was collected in fasting state from 12 adults (6 women and 6 men), it was not centrifuged and stored in -75°C. Before the examination, particular saliva samples were mixed with the use of a flow homogenizer. The content of proteins in the saliva was mean 3.0 mg/ml and pH was 6.4.

1. The evaluation of hemoglobin degradation products by salivary cathepsin D

The amount of 0.2 ml of 6% hemoglobin was added to 0.8 ml of saliva. Both hemoglobin and saliva pH was 3.5. The reaction was interrupted after 6 hours of incubation in 37°C by adding 1.0 ml of 10% trichloroacetic acid (TCA). The samples precipitated at time 0 were considered to be the controls. The following were determined in supernatant fluid (obtained by centrifugation – 10000 x g for 10 min) containing hemoglobin degradation products:

- Tyrosine and peptide bindings, using Folina and Ciocalteau method with copper modification (3)
- Tyrosine using Folina and Ciocalteau method (3)
- Alpha-amine groups using ninhydrin method (2)
- Alpha-amine groups using TNBS (1)
- Aromatic aminoacids by absorbance measurement, at 280 nm of the wave length.

2. The determination of optimal pH for cathepsin D activity

The amount of 0.1 ml of 6% hemoglobin was added to 0.4 ml of saliva (pH varied from 2.5 to 5.0 with the divisions every 0.5 of pH unit) and incubated at 37°C for 6 hours. The reaction was interrupted by adding 0.5 ml of 10% TCA. The assays precipitated at time 0 were the controls. Tyrosine and peptide bindings were determined in supernatant fluid (obtained by centrifuga-

Table 1. **The content of hemoglobin degradation products by salivary cathepsin D determined with various methods**

Products	Reagent/method	Value
Tyrosine, peptide bindings	Folina and Ciocalteau, reagent copper	248.1 ±25.0 Tyr, nmol/ml
Tyrosine	Folina i Ciocalteau	123.8 ±11.4 Tyr, nmol/ml
Alpha-amine groups	ninhydrin reagent	1.6 ±0.2 Leu, μmol/ml
Alpha-amine groups	TNBS	1.2 ±0.2 Leu, μmol/ml
Aromatic aminoacids	absorbance, 280 nm	0.632 ±0.05 $E_{280\ nm}$

Table 3. **The activity of human salivary cathepsin D estimated at various time, at 3.5 pH**

Incubation time, h	Cathepsin D activity	
	Tyr, nmol/ml	%
3	98.4 ±10.2	41.2
6	238.6 ±22.6	100.0
9	310.0 ±29.6	130.0
12	364.8 ±38.2	152.9
15	398.6 ±41.8	167.0
18	458.0 ±44.8	191.9
21	498.4 ±52.0	208.9
24	526.4 ±54.2	220.6

Table 5. **The influence of dithiothreitolu and the inhibitor of common vetch seed coats on the activity of salivary cathepsin D estimated at 3.5 pH**

Compound	Cathepsin D activity	
	Tyr nmol/ml	%
Control	182.4 ±19.2	100.0
Dithiothreitol	168.6 ±17.8	92.4
Inhibitor in common vetach seed coats	66.8 ±9.8	36.6

Table 2. **The activity of human salivary cathepsin D estimated at various pHs, during 6 hours of incubation**

pH	Cathepsin D activity	
	Tyr, nmol/ml	%
2.5	18.0 ±2.3	7.5
3.0	158.6 ±12.6	65.9
3.5	240.8 ±24.5	100.0
4.0	182.0 ±16.0	75.6
4.5	98.6 ±9.8	40.9
5.0	64.3 ±7.4	26.7

Table 4. **The activity of human salivary cathepsin D estimated at various temperatures, at 3.5 pH**

Temperature, °C	Cathepsin D activity	
	Tyr nmol/ml	%
0	0.0 ±0.0	0.0
5	0.8 ±0.1	0.3
10	4.6 ±0.5	1.9
15	16.2 ±2.3	6.8
20	94.8 ±9.6	39.7
25	160.5 ±15.2	67.0
30	182.2 ±19.3	76.0
35	239.6 ±19.8	100.0
40	236.4 ±23.0	98.7
45	140.0 ±14.2	58.4
50	46.3 ±5.2	19.3
55	12.4 ±1.4	5.2
60	0.0 ±0.0	0.0

tion – 10000 x g for 10 min) using Folina and Ciocalteau method with copper modification.

3. The determination of optimal incubation time

The amount of 0.1 ml of 6% hemoglobin was added to 0.4 ml of saliva and incubated for 3, 6, 9, 12, 15, 18, and 24 hours. Reagent pH was 3.5. The procedure was completed as in point 2.

4. The choice of incubation temperature

The amount of 0.1 ml of 6% hemoglobin was added to 0.4 ml of saliva. The mixture was incubated at 0°C to 60°C, with divisions every 5°C. The procedure was completed as in point 2.

5. The influence of dithiothreitol on cathepsin D activity

The amount of 0.1 ml of ditiotreitol (5 μmol/ml) was added to 0.3 ml of saliva and preincubated for 30 min at 37°C. Then, 0.1 ml of 6% hemoglobin was added and the procedure was completed as in point 2.

6. The influence of common vetch seed coat inhibitor on cathepsin D activity

The amount of 0.1 ml of the inhibitor (25 μmol/ml) was added to 0.3 ml of saliva and preincubated for 30 min at 37°C. Afterwards, 0.1 ml of 6% hemoglobin was added and the procedure was completed as in point 2.

Results and discussion

The products of hemoglobin degradation by cathepsin D can be determined with different methods, as it can be seen in *Tab. 1*. The results showed that the most preferable method was tyrosine and peptide bindings determination using Folina and Ciocalteau reagents and with copper reagent.

Human saliva cathepsin D presented the highest activity in reaction with hemoglobin at pH 3.5 (*Tab. 2*). As *Tab. 3* showed, the optimal incubation time was 6 hours. The highest activity of the enzyme was revealed at 35-40°C (*Tab. 4*). Dithiothreitol did not affect the salivary cathepsin D activity but the inhibitor of common vetch seed coat suppressed the enzyme activity (*Tab. 5*).

The differences of cathepsin D activities were not observed as far as the sex was concerned. The saliva used for determining the cathepsin D activity should not be centrifuged but homogenized.

The results point to the fact that cathepsin D activity in the saliva, in the oral cavity, is relatively low and its determination

required many hours of incubation and lowering salivary pH to 3.5-4.0. Thus, salivary cathepsin D does not take part in protein digestion. However, we cannot exclude its participation in organic proteolysis, i.e. splitting only single peptide bindings, which results in manifestation or loss of biological properties of a given protein [7].

In inflammatory conditions, such as sialitis, gingivitis, and oral mucositis, a significant increase in cathepsin D activity, pH decrease, and local destructive activity of cathepsin D (specifically in case of tooth pocket inflammation) can occur, which is manifested by numerous enzyme activity increase [5,6].

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Bacterial composition in the supragingival plaques of children with and without dental caries

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Abstract

Purpose: The purpose of the present investigation was to determine if the supragingival bacterial composition plaques in children with caries would differ from those found in caries-free controls.

Material and methods: Pooled supragingival plaque samples from the smooth surfaces of teeth were collected from 75 children with caries and 131 children without caries. The plaque samples were analysed for bacterial content by cultures on a series of non-selective and selective media for aerobic, microaerophilic and anaerobic bacteria. Additionally, the specimens of dentine carious lesions were examined. The standard culture procedures and identifications of bacteria were used.

Results: Among 131 children without dental caries, 41 (31.3%) were at preschool age with deciduous teeth and 90 (68.7%) at school age with permanent teeth. Dental plaques of caries-free children revealed 452 strains, out of which 326 (72.1%) were from permanent teeth, 126 (27.9%) – from deciduous teeth ($p=0.0001$). Among 75 children with dental caries, 61 (81.3%) were at preschool age and 14 (18.7%) – at school age. There were 239 strains isolated from supragingival plaques in children with dental caries, 187 (78.2%) – in preschool children, and 52 (21.8%) – in school children ($p<0.05$). From dentine carious lesions in these children, 209 strains were isolated; 164 from preschool children and 45 – from school children ($p<0.05$). Gram-positive bacteria were isolated more frequently than Gram-negative ones ($p=0.0001$) from supragingival plaques both in children with and without dental caries. *Streptococcus* genus bacteria were isolated more often ($p=0.0002$) from the plaques in school children without dental caries. The proportion (%) of aerobic and anaerobic bacteria was comparable ($p>0.05$) in dental plaques in children with and without dental caries, except for *Veillonella* spp., which were isolated more frequently from dental plaques in school children with dental caries ($p=0.01$).

Conclusions: 1) Generally, there was no statistically significant difference of bacterial species composition isolated from supragingival plaques in children with deciduous and permanent dental caries and caries-free children. 2) There was no difference between bacterial composition in dentine carious lesions of deciduous teeth and permanent teeth as compared to supragingival plaques in these children (except for *Neisseria* spp., *Peptostreptococcus* spp.).

Key words: bacterial composition, supragingival plaques, dentine carious lesions, deciduous teeth, permanent teeth, caries-free children.

Introduction

Dental plaque is a complex microbial community growing as a biofilm on enamel surfaces. The aetiology of both dental caries (tooth decay) and various forms of periodontal disease has long been recognized to be related to bacterial accumulations and plaque composition. Despite extensive analysis of plaque samples from healthy and diseased subjects as well as data derived from gnotobiotic and germ-free animal experiments, no single microbe has been identified which satisfies Koch's postulates for an infectious agent in either caries or periodontitis [1-5]. Most recent evidence suggests that both diseases have a multibacterial aetiology and therefore it is important to gain insight into the total bacterial composition of dental plaque [2-4]. It has been found that many of early microbial colonizers of human dental plaque are of great importance in the succession stages of biofilm formation and its overall effect on the oral health of the host [4].

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Table 1. **Aerobic and anaerobic bacteria in supragingival plaques and carious lesions of children**

Specimens (No. of children) / Bacteria	Supragingival plaques			Carious lesions (75)
	Caries (75)	Caries-free (131)	Total (206)	
I. Aerobic bacteria	**150**	**291**	**441**	**125**
1. Gram-positive	72	148	220	61
Gram-positive cocci	72	148	220	61
Streptococcus spp.	*47	*102	149	37
Gemella morbillorum	0	1	1	1
Staphylococcus spp.	24	41	65	22
Micrococcus spp.	1	4	5	1
2. Gram-negative	78	143	221	64
Gram-negative cocci	59	112	171	47
Neisseria spp.	59	112	171	47
Gram-negative bacilli	19	31	50	17
Haemophilus spp.	12	27	39	12
Enterobacter aerogenes	1	4	5	2
Escherichia coli	*5	*0	5	2
Pseudomonas aeruginosa	1	0	1	1
II. Anaerobic bacteria	**89**	**161**	**250**	**84**
1. Gram-positive	73	120	193	64
Gram-positive cocci	68	110	178	57
Peptococcus spp.	31	44	75	29
Peptostreptococcus spp.	37	66	103	28
Gram-positive bacilli	5	10	15	7
Actinomyces spp.	0	6	6	0
Bifidobacterium spp.	4	2	6	3
Eubacterium lentum	1	2	3	3
Lactobacillus spp.	0	0	0	1
2. Gram-negative	16	41	57	20
Gram-negative cocci	16	38	54	20
Veillonella spp.	16	38	54	20
Gram-negative bacilli	0	3	3	0
Fusobacterium spp.	0	1	1	0
Prevotella oralis	0	1	1	0
Bacteroides eggerthii	0	1	1	0
Total	**239**	**452**	**691**	**209**
Gram-positive	**145**	**268**	**413**	**125**
Gram-negative	**94**	**184**	**278**	**84**

* $p<0.05$

The purpose of the present investigation was to determine if the supragingival bacterial plaques in children with caries would differ from those found in caries-free controls.

Material and methods

Pooled supragingival plaque samples from the smooth surface of teeth were collected from 75 children with dental caries and 131 children without caries. The plaque samples were analysed for bacterial content by cultures on a series of non-selective and selective media for aerobic, microaerophilic and anaerobic bacteria. Additionally, the specimens of dentine carious lesions were examined. The standard culture procedures and identifications of bacteria with commercial kits (API Staph, API Strep, API NH, API Coryne, API 20E, API 20NE, API A) were used [3,5,6].

The chi-squared test was used to analyse the differences between the isolation frequencies (shown as percentages or number). Groups were considered significantly different from each other if $P \leq 0.05$.

The approval of the local Ethics Committee was obtained prior to the study.

Results

This study carried out in 206 children (96 males and 110 females) aged 4-18. Of which divided in two groups such as: 102 preschool children aged 4-7 (mean age =5.5 years) and 104 school children (52: 12-year-old and 52: 18-year-old). These children attended to preschool and school in Białystok.

Caries prevalence were diagnosed visually and only lesions accompanied by enamel loss or cavities involving the dentin of

Table 2. **Isolation frequency (%) of bacterial species in supragingival plaques of children without caries**

Bacterial species (No. of strains)	Preschool children (n=41)	School children (n=90)	Total (n=131)
Aerobic bacteria			
Streptococcus spp. (102)	*58.5	*86.7	77.9
S. intermedius (3)	4.9	1.1	2.3
S. mitis (22)	17.1	16.7	16.8
S. oralis (12)	7.3	10.0	9.2
S. salivarius (24)	*7.3	*23.3	18.3
S. sanguis (7)	2.4	6.7	5.3
S. vestibularis (34)	19.5	28.9	25.9
Gemella morbillorum (1)	0	1.1	0.8
Staphylococcus spp. (41)	34.1	30.0	31.3
S. aureus (23)	24.4	14.4	17.6
S. capitis (1)	0	1.1	0.8
S. epidermidis (11)	9.8	7.8	8.4
S. haemolyticus (1)	0	1.1	0.8
S. hominis (3)	0	3.3	2.3
S. warneri (2)	0	2.2	1.5
Micrococcus spp. (4)	0	4.4	3.1
Neisseria spp. (112)	85.4	85.6	85.5
N. flavescens (19)	7.3	17.8	14.5
N. mucosa (23)	19.5	16.7	17.6
N. sicca (37)	26.8	28.9	28.2
N. subflava (33)	31.7	22.2	25.2
Haemophilus spp. (27)	12.2	24.4	20.6
H. influenzae (2)	0	2.2	1.5
H. parainfluenzaae (25)	12.2	22.2	19.1
Enterobacter aerogenes (4)	2.4	3.3	3.1
Anaerobic bacteria			
Actinomyces naeslundii (6)	4.9	4.4	4.6
Bifidobacterium spp. (2)	2.4	1.1	1.5
Eubacterium lentum (2)	2.4	1.1	1.5
Peptococcus spp. (44)	34.1	33.3	33.6
Peptostreptococcus spp. (66)	48.8	51.1	50.4
Veillonella spp. (38)	21.9	32.2	29.0
Fusobacterium spp. (1)	0	1.1	0.8
Prevotella oralis (1)	0	1.1	0.8
Bacteroides eggerthii (1)	0	1.1	0.8

* $p<0.05$

the primary teeth or the secondary teeth were considered. The labial, buccal and lingual tooth surfaces were inspected visually for the presence of dental plaque, without using disclosing solution. Children with visible plaque on one or more tooth surfaces were categorized as positive (visible plaque) as well as visible caries. Pooled supragingival plaque and samples of dentine carious lesions were collected and examined bacteriologically. This results are presented in *Tab. 1*.

Among a total of 206 examined children 131 (63.6%) were without dental caries and in 75 (36.4%) caries were found. Dental plaques of caries-free children revealed 452 strains belonged to 16 genera of 7 aerobic and 9 anaerobic bacteria. From dental plaques of children with caries a total 239 strains were isolated, 8 aerobic and 5 anaerobic genera. In addition, from 75 samples of dentine carious lesions 209 strains were detected of which 9 aerobic and 6 anaerobic genera of bacteria (*Tab. 1*). Gram-positive bacteria were isolated more frequently than Gram-negative ones ($p=0.0001$) from supragingival plaques both in children with and without dental caries as well as from carious lesions.

Out of 131 children without dental caries, 41 (31.3%) were at preschool age with deciduous teeth and early mixed dentition and 90 (68.7%) at school age with permanent teeth (*Tab. 2*). A total of 326 (72.1%) bacterial strains (29 species) were from permanent teeth and 126 (27.9%) (20 species) – from deciduous teeth ($p=0.0001$). *Streptococcus* genus bacteria and *Streptococcus salivarius* species were isolated more often ($p<0.05$) from the plaques in school children without dental caries (*Tab. 2*).

Among 75 children with dental caries, 61 (81.3%) were at preschool age and 14 (18.7%) – at school age (*Tab. 3*). There were 239 strains isolated from supragingival plaques in children with dental caries, 187 (78.2%) – in preschool children (23 species), and 52 (21.8%) – in school children (17 species) ($p<0.05$). From dentine carious lesions in these children, 209 strains were isolated; 164 (78.5%) from preschool children (29 species) and

***Table 3*. Isolation frequency (%) of bacterial species in supragingival plaques and dental carious lesions of children with caries**

Bacterial species (No. of strains)	Preschool children (n=61)		School children (n=14)		Total (n=75)	
	Plaque	Carious lesion	Plaque	Carious lesion	Plaque	Carious lesion
Aerobic bacteria						
Streptococcus spp. (84)	59.0	45.9	78.6	64.3	62.7	49.3
S. intermedius (2)	0	1.6	0	7.1	0	2.7
S. mitis (21)	13.1	13.1	21.4	14.3	14.7	13.3
S. mutans (2)	1.6	1.6	0	0	1.3	1.3
S. oralis (15)	8.2	9.8	14.3	14.3	9.3	10.7
S. salivarius (13)	9.8	6.6	7.1	14.3	9.3	8.0
S. sanguis (9)	8.2	6.6	0	0	6.7	5.3
S. vestibularis (22)	18.0	6.6	35.7	14.3	*21.3	*8.0
Gemella morbillorum (1)	0	1.6	0	0	0	1.3
Staphylococcus spp. (46)	34.4	29.5	21.4	28.6	32.0	29.3
S. aureus (19)	16.4	14.7	0	0	13.3	12.0
S. caprae (1)	0	1.6	0	0	0	1.3
S. cohnii (1)	0	1.6	0	0	0	1.3
S. epidermidis (11)	11.5	4.9	7.1	0	10.7	4.0
S. hominis (5)	1.6	1.6	7.1	14.3	2.7	4.0
S. simulans (1)	0	0	0	7.1	0	1.3
S. warneri (8)	4.9	4.9	7.1	7.1	5.3	5.3
Micrococcus spp.(2)	0	0	7.1	7.1	1.3	1.3
Neisseria spp. (106)	*77.0	*59.0	85.7	78.6	*78.7	*62.7
N. flavescens (13)	11.5	4.9	7.1	14.3	10.7	6.7
N. mucosa (17)	9.8	14.7	14.3	0	10.7	12.0
N. sicca (36)	22.9	16.4	50.0	35.7	28.0	20.0
N. subflava (40)	32.8	22.9	14.3	28.6	29.3	24.0
Haemophilus spp. (24)	11.5	14.7	35.7	21.4	16.0	16.0
H. influenzae (1)	0	1.6	0	0	0	1.3
H. parainfluenzaae (23)	11.5	13.1	35.7	21.4	16.0	14.7
Enterobacter aerogenes (3)	1.6	3.3	0	0	1.3	2.7
Escherichia coli (7)	8.2	3.3	0	0	6.7	2.7
Pseudomonas aeruginosa (2)	1.6	1.6	0	0	1.3	1.3
Anaerobic bacteria						
Bifidobacterium spp. (7)	3.3	3.3	14.3	7.1	5.3	4.0
Eubacterium lentum (4)	1.6	4.9	0	0	1.3	4.0
Lactobacillus spp. (1)	0	1.6	0	0	0	1.3
Peptococcus spp. (60)	44.3	**45.9	28.6	**7.1	41.3	38.7
Peptostreptococcus spp. (65)	*49.2	*/**31.1	50.0	**64.3	49.3	37.3
Veillonella spp. (36)	**14.7	22.9	**50.0	42.9	21.3	26.7

* $p<0.05$; ** $p<0.05$

45 (21.5%) – from school children (17 species) ($p<0.05$). There was no difference between bacterial composition in dentine carious lesions of preschool children and school children as compared to supragingival plaques in these children, except for *Neisseria* spp. and *Peptostreptococcus* spp. (*Tab. 3*). These genera more often were detected in dental plaques than carious lesions of preschool children ($p<0.05$). *Peptostreptococcus* spp. more frequently was also isolated from carious lesions of school children than preschool children.

Discussion

According to the results of the present study it is concluded that, there was no statistically significant difference of bacterial species composition isolated from supragingival plaques in children with deciduous and permanent dental caries and caries--free children. There was also nodifference between bacterial composition in dentine carious lesions of deciduous teeth and permanent teeth as compared to supragingival plaques in these children, except for *Neisseria* spp. and *Peptostreptococcus* spp. These bacteria more often were isolated from supragingival plaque of preschool children (77.0% and 49.2%, respectively) than from carious lesions (59.0% and 31.1%) ($p<0.05$). *Veillonella* spp. was isolated significant frequently from dental plaques in school children with dental caries (7/14; 50.0%) than preschool children (9/61; 14.7%) ($p=0.01$) while *Peptococcus* spp. was more frequent in carious lesions of preschool children (28/61; 45.9%) than school children (1/14; 7.1%) ($p=0.017$).

Mutans streptococci and *Lactobacillus* spp. which are the main aetiologic agents of dental caries in humans [1-3,7] were observed only sporadically in preschool children. *Actinomyces* spp. was detected only in dental plaque of children without caries. The proportion of cariogenic bacteria is about 10% and was comparable ($p>0.05$) in dental plaques in children with and without dental caries in both the preschool and school children as well as in carious lesions of primary and permanent teeth.

Gram-positive bacilli other than *Lactobacillus* spp. and *Actinomyces* spp. accounted for about 60% of the total Gram-positive anaerobic rods. Some species belonging to *Bifidobacterium* spp. and *Eubacterium lentum* are also found to be associated with periodontal disease and could frequently isolated from carious lesions [1].

Most, if not all, forms of dental decay are chronic bacterial infections due to the dominance in the plaques of aciduric bacterial species such ast the mutans streptococci, lactobacilli and *Actinomyces* spp. [1-7]. The results obtained by Aamdal et al. [8] do not readily support the traditional concept of caries formation according to hypothesis that root caries is the results of acid formation by acidogenic microorganisms. Authors was not detected difference in microbial composition of dental plaque and difference in plaque pH response on sound and carious root surfaces. The plaque pH response was more pronounced in the maxilla than in the mandible for both sound and carious sites. The pH response to sucrose was the same regardless of the presence or absence of mutans streptococci [8].

Despite these finding, neither *Streptococcus* spp. nor *Lactobacillus* spp. appear to be predominant pathogens in root caries, as others have shown contradictory findings [9]. It is appears that both these bacterial groups play an important role in the initiation and development of caries together with a variety of other oral microbiota, as shown by our own results and by others [10].

Our study showed that some bacterial species which are putative respiratory pathogens [11] (e.g. *S. aureus*, *H. influenzae*, *H. parainfluenzae*, *P. aeruginosa* and *E. coli*) had colonized the supragingival plaques of preschool and school children as well as children with and without caries. The isolation proportion was about 12% and was comparable, also in the samples of carious lesions.

In conclusions, culturing organisms remains an important tool for the detection of bacteria from dental plaque and other site of oral cavity. Cultured microorganisms are required for antibiotic resistance data and for elucidation of virulence mechanism. However, cultured bacteria may rapidly alter their phenotypic characteristics in vitro, and 50% of oral microorganisms have not yet been cultured [12,13].

It will also be important to control the oral microflora for systemic reasons since strong links are being established between focal infection of oral origin and a range of systemic diseases including coronary heart disease, gastrointestinal disorders and low birth weight, apart from severe overt systemic infection [13-15]. These developments are derived from an improved understanding of the ecological nature of the microbial biofilm that is dental plaque, and of its interactions with human host [15,16].

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Oral *Candida albicans* carriage in healthy preschool and school children

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Abstract

Purpose: The purpose of the present study was to detect *Candida albicans* carriage in the oral cavity of healthy preschool and school children. The second aim was the determination of correlation between *C. albicans* occurrence and dental caries in children population.

Material and methods: The samples for mycological examinations were collected from the pharynx and supragingival plaque, and carious lesions in 102 children, aged 4-7 years (preschool children) and 104 children and adolescents, aged 12 and 18 (school children). All samples were cultured directly on Sabouraud agar medium. Isolated yeasts were identified based on API 20C AUX (bioMérieux).

Results: A total of 123 *C. albicans* strains were isolated, in which 61 (49.6%) derived from supragingival plaque, 48 (39%) – from carious lesions, and 14 (11.4%) – from pharyngeal swabs. *C. albicans* was isolated from the samples of single material in 61 children (35 – school children, 26 – preschool children) while from the rest of 29 children, *C. albicans* was isolated from two (25x) or three materials (4x). *C. albicans* was detected in 48/75 (64%) children with dental caries; the rate was statistically significantly higher as compared to the overall number of children with *C. albicans* carriage (90/206; 43.7%) ($p=0.0026$). Similar results was obtained in preschool children (38/61; 62.3% and 47/102; 46.1%, respectively) ($p=0.0449$), as in school children (10/14; 71.4% and 43/104; 41.3%, respectively) ($p=0.0336$).

Conclusions: 1) *Candida albicans* was observed in the oral cavity of healthy children with high (approximately 40%) – comparable rate in school and preschool children ($p>0.05$). 2) *C. albicans* was isolated with high comparable rate from carious lesions in preschool and school children. The statistically significant differences between the rate of *C. albicans* in carious lesions in preschool children (62.3%) and school children (71.4%) and the overall number of children with *C. albicans* carriage in the oral cavity of children in both age groups ($p<0.05$) were showed.

Key words: *Candida albicans*, oral carriage, preschool and school children, dental caries, supragingival plaques, pharynx.

Introduction

Candida albicans is frequently carried in the oral cavity without causing disease [1,2], but asymptomatic carriage may place some individuals at higher risk of complications through yeast infections if they become immunosuppressed [3,4]. Among subjects initially asymptomatic for *C. albicans* infection, clinical thrush developed only in those patients who are permanent carrier of *C. albicans* prior to developing symptoms [5,6].

Despite the potential clinical relevance of *C. albicans* carriage, little is known about carriage patterns in school children [6-10]. Hence, the purpose of the present study was to analyse *C. albicans* carriage in preschool and school-age children. The second aim of this study was to correlate the presence of *C. albicans* with dental caries prevalence in this population.

Material and methods

The samples for mycological study were collected from the pharynx and supragingival plaques, and carious lesions in 102 children, aged 4-7 (preschool children) and 104 children and adolescents, aged 12 and 18 (school children). All samples were cultured directly on Sabouraud agar medium. Isolated yeasts

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Table 1. **Prevalence of *Candida albicans* in oral cavity depending on the age group of children**

	Supragingival plaques n=206	Carious specimens n=75	Pharynx n=206	Total
Preschool children (102)*	26	38	8	72
4-5 years (52)	18	19	6	43
6-7 years (50)	8	19	2	29
School children (104)	35	10	6	51
12 years (52)	16	7	3	26
18 years (52)	19	3	3	25
Total (206)	61	48	14	123

* Number of children

Table 2. **Frequency of *Candida albicans* carriage of different group children with and without caries**

Children	Total	Caries	Non-caries	p-value
Preschool children	102/47*	61/38	41/9	0.0001
Female	47/23	28/16	19/7	>0.05
Male	55/24	33/22	22/2	0.0000
4-5 years	52/26	31/19	21/7	0.0479
Female	24/12	13/7	11/5	>0.05
Male	28/14	18/12	10/2	0.0180
6-7 years	50/21	30/19	20/2	0.0002
Female	23/11	15/9	8/2	>0.05
Male	27/10	15/10	12/0	0.0016
School children	104/43	14/10	90/33	0.0140
Female	63/25	6/4	57/21	>0.05
Male	41/18	8/6	33/1	>0.05
12 years	52/22	9/7	43/15	0.0458
Female	11/4	1/1	10/3	>0.05
Male	41/18	8/6	33/12	>0.05
18 years	52/21	5/3	47/18	>0.05
Female	52/21	5/3	47/18	>0.05
Male	0	0	0	
Total	206/90	75/48	131/42	0.0000
Female	110/48	34/20	76/28	0.0317
Male	96/42	41/28	55/14	0.0000

* No. of children/ No. of *C. albicans*

were identified based on API 20C AUX (bioMérieux). The procedures for mycological studies were described previously [11]. After we had obtained informed consent from the parents and/or the guardians of the children as well as the children's own assent to participate, children were screened for health and dental disease. None of the children had clinical signs of oral candidiasis, and all were free of systemic disease.

The Ethics Committee of the Medical University of Białystok approved the study protocol.

Statistics

The prevalence of *Candida albicans* carriage was calculated as a function of age and gender of children. The differences in *C. albicans* colonization between preschool and school children were evaluated using Chi-squared test. The levels of significance were fixed at $P \leq 0.05$.

Results

A total of 123 *C. albicans* strains were isolated, in which 61 (49.6%) derived from supragingival plaques, 48 (39.0%) – from carious lesions, and 14 (11.4%) – from pharyngeal swabs (*Tab. 1*). *C. albicans* was isolated from the samples of single material in 61 children (35 – school children, 26 – preschool children) while from the rest of 29 children, *C. albicans* was isolated from two (25x) (17 – preschool, 4 – school) or three specimens (4x) only from preschool children with caries.

Candida albicans carriage was observed in a total of 90/206 (43.7%) examined children (*Tab. 2*). The difference in the frequency of *C. albicans* carriage between preschool and school children was not significant (46.1% vs 41.3%) (p=0.493) as well as between 4-5 years and 18 years (50.0% vs 41.2%) (p=0.3687). *C. albicans* was isolated with high comparable rate from carious lesions in preschool (62.3%) and school children (71.4%) (p=0.5208). No difference was seen between

non-caries preschool (21.9%) and school children (36.7%) (p=0.0943). The statistically significant differences were observed between *C. albicans* carriage in caries and non-caries groups of preschool and school children (*Tab. 2*). With the exception of 18-years-old group with caries and non-caries in which the differences was comparable (60.0% vs 38.3%) (p>0.05). The difference in the frequency of *C. albicans* carriage between preschool girls (36.8%) and boys (9.1%) was significant (p=0.0323) (*Tab. 2*).

Candida albicans was detected in 48/75 (64.0%) children with dental caries; the rate was statistically significantly higher as compared to the overall number of children with *C. albicans* carriage (90/206; 43.7%) (p=0.0026). Similar results was obtained in preschool children (38/61; 62.3% and 47/102; 46.1%, respectively) (p=0.0449), as in school children (10/14; 71.4% and 43/104; 41.3%, respectively) (p=0.0336).

Discussion

The study indicates that host age is a determining factor in yeast carriage [1,6,7,9,10,12-14]. From the neonatal period, humans go through several dentition periods, and the emergence and substitution of teeth and changes in living habits greatly change the environment of the oral cavity, and therefore influence colonization by oral commensal organisms, certainly including *Candida* spp. [12]. Russell and Lay [13] showed that the frequency of oral yeast carriage at birth was low, doubled by the time that infants were discharged from the hospital at about seven-day old, and increased sharply after one month old. Kleinegger et al. [14] demonstrated that the frequency of oral yeast carriage was 44% of the examined individuals in a group aged from 0.5 to 1.5, 24% in a 5-7-year-old group, 40% in a 15- to 18-year-old group.

Starr et al. [6] investigated the prevalence of oral *C. albicans* in children aged 8-11 at baseline (before dental treatment), post-treatment, and 12, 24, 36 months post-baseline, respectively, 47, 21, 27, 28% of children were positive.

In a review by Odd in 1988 [1], the highest reported frequencies were 71% of school children in the United Kingdom, and 56% of children in Israel.

Recently, Qi et al. [12] showed that the yeast carriage frequency is very low in the neonatal group in China, just 7.5%, which accords with the 5.7% in the study by Russel and Lay [13]. The highest frequency (70.0%) of carriage of yeast and *C. albicans* was in the primary dentition group (3-5 years) of China children [12]. The authors observed, with increasing age, the frequency of *C. albicans* decreases (6-8 years – 56.4%, early mixed dentition; 12-14 years – 49.1%, late mixed dentition and 18-21 years – 60.0%, secondary dentition) [12]. However, the frequency of yeast carriage does not decreases, because there was a large proportion of individuals in 18-21-year group with *Candida glabrata* isolated [12].

Our study did not showed the differences in the frequency rate of oral *C. albicans* carriage according to age of children; positive *C. albicans* was high from 41.2% in 18-year-old to 50% in 4-5-year-old.

In the school children described in this report, *C. albicans* carriage occurred far more frequently (41.3%) than the overage carriage rate of 10-15% reported in literature [1]. *C. albicans* prevalence in healthy school children Portuguese population was as high as 47% at baseline and decreased to 27% during 3 years, even with regular dental care [6].

The above presented results indicate that the frequency of oral yeast, mainly *Candida albicans* species, is different in different-aged children. *Candida albicans* is the most important commensal organism in the oral cavity, and the yeast carriage frequency varies not only by the age but also according to geographical area. It is therefore a reasonable possibility that these changes in frequency may be due to physiological changes related to age: changes relating to body fluids, changes at mucosal surfaces, changes related to natural barriers against yeast colonization, and changes in the living environment and habits of the individual and to the ecological environment of the oral cavity.

Different sampling and identification methods for *Candida* spp. would also certainly influence the results [1,2,6]. Because of the uneven distribution of *C. albicans* throughout the oral cavity, swab samples can yield false-negative culture more often than oral rinse samples or imprint culture [1]. The number of false-negative cultures was reduced by the swabbing of two sites rather than one [1,6]. According to our study, the frequency of *C. albicans* carriage was only 29.6% for a single samples tested and increased to 41.7% for two swab samples (p=0.01) or to 43.7% when three samples evaluated (p=0.003).

Moreover, other risk factors for *C. albicans* carriage, such as recent or current antibiotic use, were not taken into account [1].

Apart from bacteria, the importance of the presence of yeasts in the oral cavity and the incidence of dental caries have been demonstrates for adults and for children [6-10]. Our own studies revealed that the oral cavity of children with healthy teeth is almost devoid of *Candida albicans*. *Candida albicans* species only from children with caries have been detected in 64.0% (48/75) compared to 32.1% (42/131) from children caries-free (p=0.0000); it is concerning the preschool and school children.

Since caries prevalence is associated with *C. albicans* carriage [1,6-9], the eligibility requirement of the parent study of a carious lesions in at least one deciduous or permament tooth may have elevated these carriage rates in comparison to the general Polish preschool and school population.

In conclusion, our observations indicate that there is an increased risk of dental caries with *C. albicans* carriage rates in preschool and school children.

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Prevalence rate and antibiotic susceptibility of oral viridans group streptococci (VGS) in healthy children population

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Abstract

Purpose: The aim of this study was to evaluated the prevalence rate of oral viridans group streptococci (VGS) and their susceptibilities to some antibiotics in healthy children.

Material and methods: Samples of pharyngeal swabs and supragingival dental plaques for microbiological studies were collected from 206 healthy children, aged 4-18 years. Additionally, 75 samples of carious lesions from children with dental caries were included. The streptococci were isolated and identified using standard methods and commercial identification kits. For performance of antibacterial susceptibility testing of VGS strains disk diffusion and/or breakpoints procedures were used according to NCCLS standards and criteria. A total of 425 VGS strains were tested against penicillin, ampicillin, erythromycin, clindamycin, tetracycline, doxycycline, ciprofloxacin and vancomycin.

Results: A total of 239 VGS strains belonging to 8 species from pharyngeal swabs of 192 (93.2%) children were isolated. VGS strains from supragingival plaques were isolated in 149 (72.3%) healthy children ($p<0.05$), and from carious lesions in 37 (49.3%) children with dental caries. VGS strains of *S. mitis* species were isolated most frequently from 4-5 year old as compared to 12 and 18 year old children ($p<0.05$), while *S. vestibularis* strains isolated most often in 12 year old ones ($p<0.05$). Among 425 VGS strains, high level of penicillin resistance (MIC≥2.0 mg/L) was shown in 71 (16.7%) strains, 33 (46.5%) of them belonged to *S. mitis* species. VGS strains were also resistant to erythromycin (23.5%), clindamycin (23.1%), tetracyclines (T-52%, DOX-16%), gentamycin (25.9%) and ciprofloxacin (55.2%). All VGS strains were vancomycin – susceptible.

Conclusions: 1. In the oral cavities of healthy children, approximately 98% of streptococci belonged to two VGS groups, i.e. mitis and salivarius groups. Streptococci of mutans and anginosus groups were isolated sporadically (2%). 2. We observed difference in susceptibility to penicillin and other antibiotics between the various species of viridans groups streptococci. Mitis group strains (except *S. pneumoniae*) were more frequently penicillin-resistant (23%) in comparison to salivarius group of VGS strains (9%) ($p=0.0001$).

Key words: viridans group streptococci, penicillin resistance, healthy children, pharyngeal swabs, supragingival plaques, dental caries, multidrug resistance.

Introduction

The aerobic oropharyngeal or the oral cavity microbiota consists predominantly of viridans group streptococci (VGS), which play an important role in inhibiting colonization of pathogens. VGS are normal inhabitans of the oral cavity, gastrointestinal tract, and female genital tract, and they are often considered to be contaminants when isolated from blood cultures, where they may be found as transients in the bloodstream [1-3]. However, their presence may be associated with infective endocarditis, especially in patients with prosthetic heart valves, where *S. sanguis*, *S. mitis*, *S. oralis* and *S. gordonii* being frequently isolated [1,4], which together with *S. pneumoniae* are belonged to the mitis group [3,5]. Members of the mutans group streptococci are associated with dental caries in humans and animals, where *S. mutans* and *S. sobrinus* being the species most frequently isolated from carious lesions and dental plaques. *S. mutans* may also be isolated from patients with endocarditis

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Table 1. **Occurrence of viridans group streptococci (VGS) strains in throats and their resistance to penicillin (% or number of strains)**

Species \ Age (years) (No. of children)	4-5 (n=52)	6-7 (n=50)	12 (n=52)	18 (n=52)	Total (n=206)
S. anginosus	1 (1)	0	0	0	1 (1)
S. mitis	26 (38.5)	12 (41.7)	12 (16.7)	15 (13.3)	65 (29.2)
S. mutans	2 (2)	0	0	0	2 (2)
S. oralis	13 (30.8)	6 (1)	10 (20.0)	7 (0)	36 (19.4)
S. salivarius	16 (18.7)	11 (9.1)	15 (20.0)	19 (10.5)	61 (14.7)
S. sanguis	4 (0)	6 (1)	5 (0)	3 (0)	18 (5.6)
S. vestibularis	16 (6.2)	9 (0)	20 (0)	9 (1)	54 (3.7)
S. pneumoniae	2 (0)	0	0	0	2 (0)
Streptococcus spp.	80 (23.7)	44 (18.2)	62 (11.3)	53 (9.4)	239 (17.2)

[2,3]. *S. intermedius* is often isolated among the polymicrobial flora of deep abscesses, notably in the liver and brain [6]. Other member of the anginosus group may be isolated from oral abscesses, *S. anginosus* also isolated from smears of female genital tract infections [3].

VGS are the major pathogens found in non-intravenous drug users with native valve infective endocarditis [1,7,8], and are also common pathogens causing septicaemia in patients with haematological disease who receive chemotherapy and develop neutropenia [1,7-9]. The major species causing infections in neutropenic (immunocompromised) patients are *S. oralis*, *S. mitis*, *S. salivarius* and *S. anginosus* [3,10,11]. Complications associated with bacteremia in these patients include endocarditis, acute respiratory distress syndrome (ARDS) and shock [3,9,12].

In patients with haematological disease and neutropenia, oral ulcerations related to chemotherapy may develop, with the result that VGS can penetrate easily from the oral cavity into the bloodstream and cause septicaemia [8]. Antibiotic prophylaxis, especially with ciprofloxacin, has reduced the number of episodes of septicaemia caused by Gram-negative bacteria, but it has been suggested that this has increased the frequency of septicaemia caused by VGS [8,13]. Several studies have found reduced susceptibility to penicillin in VGS from such patients [8,11,13,14], with the frequency of penicillin resistance (MIC>2.0 mg/L) in isolates of VGS being as high as >40% [15].

However, recent studies have indicated that VGS are increasingly becoming resistant to many antibiotics not only to penicillin, to macrolides and others [3,5,8,11,13-16].

The aim of this study was to evaluate the prevalence of oral viridans group streptococci (VGS) and their susceptibilities to some antibiotics in healthy children.

Material and methods

Pharyngeal and supragingival dental plaque specimens were collected from 206 healthy children aged between 4 and 18 years. The specimens obtained were directly inoculated onto Mueller-Hinton agar plates with 5% defibrinated sheep blood. Plates were incubated at 37°C for 18-24h in 5% CO_2. Putative streptococcal colonies were chosen based on their morphology (β, α or γ haemolysis) and were further confirmed by compatible Gram stain and catalase negative tests [17]. These isolates further differentiated by API STREP or ID 32 STREP using API Expression automated for interpreted test strips (bioMérieux). Asignment of isolates to VGS group species was carried out according to the criteria of Facklam [5] and Ruoff et al. [3]. Additionally, samples of carious lesions from 75 children with dental caries were studied.

A total of 425 VGS strains isolated from oral cavity of 206 children were tested for antibacterial susceptibilities. For performance of antibacterial susceptibility testing of VGS strains disk diffusion and/or breakpoints procedures were used according to NCCLS (National Committee for Clinical Laboratory Standards) standards and criteria [17-20]. The VGS strains were tested against penicillin, ampicillin, erythromycin, clindamycin, tetracycline, doxycycline, ciprofloxacin and vancomycin. *S. aureus* ATCC 29 213, *S. aureus* ATCC 25 923 and *S. pneumoniae* ATCC 49 150, were used for assay control.

Statistical comparisons of the susceptibility rates and incidence of viridans group of streptococci were performed by chi-square test.

This study was approved by Ethics Committee of the Medical University of Białystok.

Results

A total of 239 VGS strains belonging to 8 species were isolated from pharyngeal swabs of 192/206 (93.2%) children (*Tab. 1*). One species of VGS strains was observed in 147 (76.6%) pharyngeal swabs, two species in 43 (22.4%) and three species in 2 (1%) pharyngeal swabs. No VGS strains isolated in 14 (6.8%) children. VGS strains belonged to 8 species were isolated most frequently from 4-5 year old children (80/239; 33.5%) as compared to 12 year old children (62/239; 25.9%) ($p<0.05$). We noticed that there was no growth of *S. anginosus*, *S. mutans* and *S. pneumoniae* species from the throat of children aged 6-7, 12 and 18 years old (*Tab. 1*). VGS strains of *S. mitis* species were isolated most frequently from 4-5 year old children (26/52; 50%) as compared to 6-7 years old (12/50; 24%) ($p<0.05$), young aged 12 (12/52; 23.1%) ($p<0.05$) and 18 year old (15/52; 28.8%) ($p<0.05$).

Table 2. **Occurrence of VGS strains in supragingival dental plaques and their resistance to penicillin (% or number of strains)**

Species \ Age (years) (No. of children)	4-5 (n=52)	6-7 (n=50)	12 (n=52)	18 (n=52)	Total (n=206)
S. intermedius	0	2 (0)	1 (0)	0	3 (0)
S. mitis	6 (4)	9 (2)	9 (2)	9 (1)	33 (27.3)
S. mutans	1 (1)	0	0	0	1 (1)
S. oralis	4 (2)	4 (1)	8 (0)	3 (0)	19 (15.8)
S. salivarius	5 (0)	4 (1)	9 (1)	13 (7.7)	31 (9.7)
S. sanguis	3 (1)	3 (0)	5 (0)	1 (0)	12 (8.3)
S. vestibularis	6 (2)	13 (0)	17 (0)	14 (0)	50 (4.0)
Streptococcus spp.	25 (40.0)	35 (11.4)	49 (6.1)	40 (5.0)	149 (12.8)

Table 3. **Occurrence of VGS strains in carious lesions and their resistance to penicillin (number of strains)**

Species \ Age (years) (No. of children)	4-5 (n=31)	6-7 (n=30)	12 (n=9)	18 (n=5)	Total (n=75)
S. intermedius	0	1 (0)	1 (0)	0	2 (0)
S. mitis	3 (3)	5 (2)	1 (0)	1 (0)	10 (5)
S. mutans	0	1 (1)	0	0	1 (1)
S. oralis	1 (1)	5 (1)	2 (0)	0	8 (2)
S. salivarius	3 (0)	1 (0)	1 (0)	1 (1)	6 (1)
S. sanguis	2 (1)	2 (0)	0	0	4 (1)
S. vestibularis	2 (1)	2 (0)	2 (0)	0	6 (1)
Streptococcus spp.	11 (6)	17 (4)	7 (0)	2 (1)	37 (11)

Table 4. **Incidence rate (%) of penicillin resistant VGS strains from oral cavity in healthy children**

Group and species (No. of strains)	Categories of susceptibility: Susceptible (S) *(≤0.06)	Intermediate (I) *(0.12-1.0)	Resistant (R) *(≥2)
Salivarius group			
S. salivarius (98)	29.6	57.1	13.3
S. vestibularis (110)	48.2	47.3	4.5
Mitis group			
S. mitis (108)	13.9	55.6	30.5
S. oralis (63)	34.9	46.0	19.1
S. sanguis (34)	32.4	28.8	8.8
S. pneumoniae (2)	**2		
Anginosus group			
S. anginosus (1)			1
S. intermedius (5)	3	0	2
Mutans group			
S. mutans (4)			4
Total (425) *Streptococcus* spp.	31.8	52.4	16.7

* MIC in mg/L according to NCCLS; ** No. of strains

Penicillin high level resistance (MIC≥2.0 mg/L) was observed in 17.2% (62/239) of VGS strains isolated from pharyngeal swabs and most frequently seen in *S. mitis* species (19/65; 29.2%) of preschool and school children (*Tab. 1*).

Similar analysis regarding the incidence rate of VGS strains from supragingival plaques of children in different ages and resistance to penicillin are shown in *Tab 2*. Compared to pharyngeal swabs no *S. anginosus* and *S. pneumoniae* species isolated from supragingival plaques, in addition 3 strains of *S. inermedius* species were isolated from supragingival plaques. In general, fews of VGS strains belonged to one species isolated from supragingival plaques (149/206; 72.3%) as compared to pharyngeal swabs ($p<0.05$). The prevalence of resistance to penicillin (19/149; 12.8%) was comparable ($p>0.05$) in both VGS strains isolated from supragingival plaques and from pharyngeal swabs (*Tab. 1* and *2*).

VGS strains from carious lesions from children with dental caries were less frequently isolated (37/75; 49.3%) (*Tab. 3*), where the highest incidence of resistance to penicillin was seen among them (29.7%). Similar to the other results (*Tab. 1* and *2*), *S. mitis* species isolated from the carious lesions was most frequent resistance to penicillin (10/37; 27%).

Tab. 4 represent the results of penicillin susceptibility among a total 425 VGS strains isolated from the oral cavity of 206 healthy children. High level resistance to penicillin (MIC≥2.0 mg/L) was shown in a total of 71 (16.7%) strains. Resistance to penicillin was observed only in 18 (8.7%) from 208 strains of salivarius group; resistance occurred more frequently in *S. salivarius* (13/98; 13.3%) than in *S. vestibularis* species (5/110; 4.5%) ($p<0.05$) (*Tab. 4*).

In 205 isolated strains of mitis group (except *S. pneumoniae*) resistance to penicillin was appeared in 48 (23.4%) of them. Highest level of penicillin resistance was observed in the *S. mitis* isolated strains (33/108; 30.5%), and the results was similar to the resistance in *S. oralis* (12/63; 19.1%) ($p>0.05$). Resistance to penicillin among *S. sanguis* species was less significant (3/34;

Table 5. **Incidence rate (%) of resistant VGS strains to penicillin and other antibiotics**

Group and species (No. of strains)	Antibiotics							
	P	AM	E	CC	T	DOX	GM	CIP
Salivarius group								
S. salivarius (98)	13.3	3.1	29.6	23.5	51.0	16.3	26.5	53.8
S. vestibularis (110)	4.5	4.5	10.0	11.8	45.5	7.3	15.5	43.7
Mitis group								
S. mitis (108)	30.5	26.9	43.5	42.6	77.8	31.5	34.3	65.1
S. oralis (63)	19.1	12.7	9.5	15.9	36.5	11.1	27.0	56.6
S. sanguis (34)	8.8	8.8	11.8	8.8	35.3	8.8	35.3	64.5
S. pneumoniae (2)	0	0	0	0	0	0	0	0
Anginosus group								
S. anginosus (1)	1*	1	1	1	1	0	1	1
S. intermedius (5)	2	1	2	2	1	0	3	1
Mutans group								
S. mutans (4)	4	4	0	0	0	0	0	4
Total (425) *Streptococcus* spp.	16.7	11.5	23.5	23.1	52.0	16.0	25.9	55.2

P – penicillin; AM – ampicillin; E – erythromycin; CC – clindamycin; T – tetracycline; DOX – doxycycline; GM – gentamycin; CIP – ciprofloxacin;
* No. of resistant strains

8.8%) compared to *S. mitis* (p=0.021). Only in 3/6 isolated strains of anginosus group resistance to penicillin was observed. Resistance to penicillin was observed also in all of *S. mutans* isolated strains (*Tab. 4*).

Resistant to erythromycin and clindamycin was seen in 98/425 (23.1%) and 100/425 (23.5%) of VGS isolated strains, respectively; most frequent in *S. mitis* and *S. salivarius* species (*Tab. 5*). Like for penicillin, particularly a significant different in resistant to erythromycin and clindamycin between mitis group (57/205; 27.8% and 59/205; 28.8%, respectively), and salivarius group (40/208; 19.2% and 36/208; 17.3%) (p=0.0399 and p=0.0056) was seen. Considerably high percent of resistance observed to tetracyclines, gentamycin and ciprofloxacin. All VGS strains were vancomycin – susceptible.

Discussion

Antimicrobial resistance among the viridans group of streptococci (VGS) has emerged as a hindrance to effective antibiotic therapy [5,8,11,14-16].

Penicillin resistance in viridans group streptococci (VGS) has been described since the 1970s [21], although the incidence has gradually increased. Penicillin resistance rates of >40% for VGS are common [11,14,15,22-24], partieularly for *S. mitis* and *S. sanguis*. Recently, Husain et al. [24] have shown that *Streptococcus mitis* accounted for 58% of invasive viridans streptococcal infections in children with malignancies of which 51% were penicillin-nonsusceptible (resistant). There was no significant association between species or penicillin susceptibility pattern and clinical presentation or outcome [24].

Rotimi et al. [25] evaluated the prevalence of antibiotic-resistant VGS in healthy children. Of the 540 VGS isolates from 102 children, 58% were from the tooth surfaces and 42% from the tongue. The most prevalent were *S. salivarius* (21.5%) and *S. sanguis* (16.3%). Resistance rate to penicillin was 15.9%. The data authors [25] showed species-related and site-related variations in the susceptibility pattern. At the species level, 26% and 23% of *S. salivarius* and 23% and 14% of *S. mutans* from the tooth and tongue, respectively were resistant to penicillin [25].

Our objective also was to evaluate the prevalence of antibiotic-resistant VGS in healthy children. Of the 425 VGS isolates 56.2% were from the throat swabs, 35.1% from the supragingival plaques, and only 8.7% from the dentine carious lesions (mainly from preschool children: 28/37; 75.7%). The most prevalent were: *S. vestibularis* (25.9%), *S. mitis* (25.4%), *S. salivarius* (23.1%) and *S. oralis* (14.8%). Among all VGS strains resistance rate to penicillin was 16.7%. Our data, similar to Rotimi et al. [25] results and show species-related and site-related variations in the susceptibility to penicillin and an emerging high prevalence of penicillin-resistant VGS. For example, at the species level, 29%, 27% and 50% of *S. mitis* and 4%, 4% and 17% of *S. vestibularis* from the pharynx, supragingival plaque and caries lesions, respectively were resistant to penicillin.

Among other classes of agents, resistance in VGS has been described for macrolides, lincosamids, tetracyclines, quinupristin-dalfopristin and fluoroquinolones [3,5,11,13,14,16,22,25,26]. The incidence of resistance to most drugs, except for the macrolides [14,16,22,23,25], usually was low. Malhotra-Kumar et al. [16] showed macrolide-resistant VGS in >70% from pharyngeal swabs of the healthy Belgian population (17-25 years old). Half macrolide-resistant isolates were *S. mitis*, while the other half were distributed among eight different VGS species. Penicillin resistance was observed in only one macrolide-resistant isolate according to early mentioned authors [16]. In contrasts with recent data on VGS where comparable levels of penicillin and macrolide resistance have been observed [14].

Our results showed that the resistance rates to erythromycin and clindamycin were 23.5% and 23.1%, respectively. Rotimi et al. [25] observed the resistance to erythromycin and clin-

damycin respectively in 32.6% and 15.4% strains of VGS from healthy children. High level resistance to tetracycline, gentamycin and ciprofloxacin was observed among VGS strains in our study (52%, 25.9% and 55.2%, respectively). All strains of VGS tested by us and Rotimi et al. [25] and others [3,5,14,22,23] were sensitive to vancomycin. Single report of resistance to vancomycin has been noted for *Streptococcus mitis*, only [27].

Conclusions

In conclusion, a high frequency of penicillin resistance in oral isolates of VGS and its co-resistance to erythromycin, clindamycin, tetracycline, gentamycin and ciprofloxacin among healthy children was observed. The data showed species-related and site-related variations in the susceptibility patterns.

There are some important reasons why clinical microbiologists should identify viridans streptococci to a level species:

1. To delineate the spectrum of disease caused by specific species; for example bacteremia caused by *Streptococcus anginosus* (known as *S. milleri*) is associated with deep visceral abscesses (see "Introduction")
2. To differentiate between therapeutic failure and reinfection. Patients with recurrent endocarditis may not have been adequately treated during a previous episode if the same strain is isolated or may present with a new infection and a new strain might be anticipated. By identifying the organism fully, this distinction can often be made.
3. Monitoring the emergence of antibiotic resistance. Strains of *Streptococcus mitis* have been noted to be more resistant to penicillin for instance. In patients with underlying neutropenia, where antibiotic-resistant VGS might itself cause life-treatening infections.
4. In healthy individuals, where antibiotic-resistant VGS might transfer the resistance determinants to pathogenic streptococci such as β-hemolytic streptococci (e.g. *S. pyogene*s, *S. agalactiae* and other) and *Streptococcus pneumoniae*. Viridans group streptococci (VGS) are gaining significance as reservoirs of resistance determinants for respiratory tract pathogens [3,5,16,23,25].

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The relationship between masticatory efficiency and the state of dentition at patients with non rehabilitated partial lost of teeth

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Abstract

Factors believed to affect masticatory efficiency include loss of postcanine teeth, bite force, severity of malocclusion, occlusal contact area, body size and oral motor function.

The aim: to record if there is relationship between masticatory efficiency and the state of dentition at patients whose occlusion has never been rehabilitated.

Material: The study was performed in 22 patients who were missing over 50% of their functional dental units and never used any prosthetic appliances and in 15 healthy completely dentate controls.

Methods: The masticatory efficiency was measured using Optosil test for 20 and 80 cycles of chewing. The occlusal conditions were analyzed by means of the computerized T-Scan II System which registered the maximal force of pressure during the maximal occlusal contacts, the time which passed between the first contact and the maximal force of pressure and the occlusal platform area.

Results: It was observed a considerable difference in the integrity of the masticatory system between both groups. The force of pressure on the indicator, chewing platform area and the time from the first contact to the maximal force calculated in T-Scan II System differs significantly between both groups. The value of X_{50} for 20 and 80 cycles of chewing estimated in Optosil test were statistically significant only for 80 cycles of chewing.

Conclusion: The severe reduction of the number of functional dental units is caused of the impairment of chewing ability but prolongation of mastication could improve the comminution of hard food.

Key words: state of dentition, masticatory efficiency, Optosil test, T-Scan II System.

Introduction

The action of chewing food represents the initial step of its processing for digestion and absorption in the next part of digestive tract. The loss of teeth as a consequence of oral pathology, trauma or hereditary missing teeth results in the less or more advanced impairment of masticatory function. Although the masticatory system is easy to examine and a lot of different authors referred to the problem of chewing, there are still many aspects that need explanation to understand. Factors believed to affect masticatory efficiency include loss of postcanine teeth, bite force, severity of malocclusion, tactile sensitivity, occlusal contact area, body size and oral motor function [1-8]. The best predictor of masticatory performance without using complicated devices is the number of postcanine functional dental units, which is subsequently connected with the force of biting [9]. The main force of crushing food is localized in the posterior region of teeth while the centre of the occlusal contacts is located in the first molar regions (10). It was suggested that a larger maximal occlusal force was connected with a higher masticatory performance [11]. As well as the influence of occlusal contact area on chewing ability is not univocal, it could be found the contradictory results [7,12].

The aim of this investigation is to find out whether there is relationship between masticatory efficiency and the state of dentition at patients whose occlusion has never been rehabilitated.

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Table 1. **The number of teeth and functional dental units* in the examined and the control group. Means and ± SD are reported**

Dental characteristics	Examined group (n=22)		Control group (n=15)	
	Mean	SD	Mean	SD
Number of teeth	12	7	28	0
Functional dental units	4	3	14	0
Anterior functional dental units	3	2.5	6	0
Premolar functional dental units	0.6	0.8	4	0
Molar functional dental units	0.1	0.3	4	0

* The functional dental unit = a pair of any opposing teeth

Table 2. **Occlusal analysis recorded in T-Scan II System in the examined and the control group. Means and ± SD are reported**

T-Scan	Examined group (n=22)		Control group (n=15)		p
Parameters	Mean	SD	Mean	SD	
F	1799	2273	10041	7581	<0.001
CPA	45.9	54.8	252.9	112.8	<0.001
T	0.6	0.64	2.29	1.2	<0.001

F – the force of pressure on the indicator; CPA – chewing platform area – number of pixels multiplied by 1.6 mm^2 (the size of pixel); T – the time from the first contact of teeth to the maximal force (s)

Material and methods

The data was obtained in the group of 22 patients 39-61 years of age (average 51+/-7). They were 12 women and 10 men with the lack of teeth above 50% of so called functional dental units (a functional dental unit is a pair of opposite monomial teeth). The period they had lacked teeth was no shorter than one year. Their occlusion was never rehabilitated and they had not suffered from any diseases of the stomatognathic system. It can be said their health was in a good condition.

The study was also performed in 15 healthy completely dentate (that is 28 teeth, 14 dental functional units) volunteers as the controls. The group was composed of 9 women and 6 men corresponding the examined group with age (38-56, in average 49+/-4). They had not suffered from any diseases.

The people in both groups were selected on the basis of dental examination which included a comprehensive dental assessment, when the number of functional dental units was established, evaluation of masticatory efficiency and registration of occlusion in central position.

Masticatory efficiency:

The masticatory efficiency was measured using Optosil test for 20 and 80 cycles of chewing. Chewed test food was sieved through a stack of 10 sieves with aperture between 5.5 and 0.5 mm. The distribution of particle sizes by weight of the comminuted test food was described according to a Rosin-Rammler equation. The parameter X_{50} for 20 and 80 cycles of chewing by means the aperture of a theoretical sieve through which half of the weight could pass was calculated for a statistical analysis [12].

T-Scan analysis

The T-Scan allows quantification of occlusal contact data. The system consists of a sensor and a support, the handle assembly, the processing unit, software and a built in printer. When the patient closes firmly on the sensor, the resultant reduction in electric resistance is translated into an image on the screen. It allows its operator to record parameters such as bite length and the number, distribution, timing and relative force of teeth contacts [13,14]. We also calculated the size of chewing platform area of teeth during the pressure on the base of occlusal contact distribution.

The protocol study has been approved by the institutional Bioethic Commission and a written informed consent of each patient was obtained.

Statistical analysis

A descriptive analysis of each variable was made after calculating its frequency distribution and characteristic parameters. The statistical differences between the parameter values were tested by U Mann-Whitney test. The significance level was set at 0.05. The analysis was made using Statistica 6.0 Package.

Results

The *Tab. 1* shows the comparison between the examined and control groups with regard to the number of teeth and functional dental units. Functional dental units were subdivided by position in the dental arch: molar functional dental units (maximum 4), premolar functional dental units (maximum 4) and anterior functional dental units (maximum 6) [9]. On the base of this table we can observe a considerable difference in the integrity of the masticatory system between both groups. The average number of functional dental units was only four in the examined group and they were mainly anterior units.

The force of pressure on the indicator, chewing platform area and the time from the first contact to the maximal force calculated in T-Scan II System are presented in the *Tab. 2*.

Table 3. **Values of X_{50} (mm) established in Optosil test in the examined and the control group. Means and ± SD are reported**

Parameter	Examined group (n=22)		Control group (n=15)		p
	Mean	SD	Mean	SD	
X_{50}/20	5.27	0.31	5.08	0.31	n.s.
X_{50}/80	4.95	0.74	3.76	1.00	<0.001

X_{50} (mm) – the aperture of a theoretical sieve, through which half of the weight could pass; 20, 80 – the number of chewing strokes; n.s. – non significant

Table 4. **Correlations between estimated parameters for the whole participants**

Parameter	F	CPA	f.d.u.	X_{50}/ 20
F				
CPA	r=0.9530 **p<0.001**			
f.d.u.	r=0.6568 **p<0.001**	r=0.8138 **p<0.001**		
X_{50}(20)	r=-0.2344 **p=0.163**	r=-0.2622 **p=0.117**	r=-0.2816 **p=0.091**	
X_{50}(80)	r=-0.3920 **p=0.016**	r=-0.4983 **p=0.002**	r=-0.6308 **p<0.001**	r=0.7384 **p<0.001**

F – the force of pressure on the indicator (T-Scan II analysis) CPA – chewing platform area – number of pixels multiplied by 1.6 mm^2 (the size of pixel) (T-Scan II analysis); $T_{1/2}$ – the half time of gastric emptying in the ^{13}C octanoid acid breath test (min); f.d.u. – the number of functional dental units
X_{50}(20) – the aperture of a theoretical sieve through which half of the weight could pass for 20 strokes of chewing in Optosil test (mm)
X_{50}(80) – the aperture of a theoretical sieve through which half of the weight could pass for 80 strokes of chewing in Optosil test (mm)

Figure 1. **The value of X_{50} for 20 cycles of chewing estimated in Optosil test**

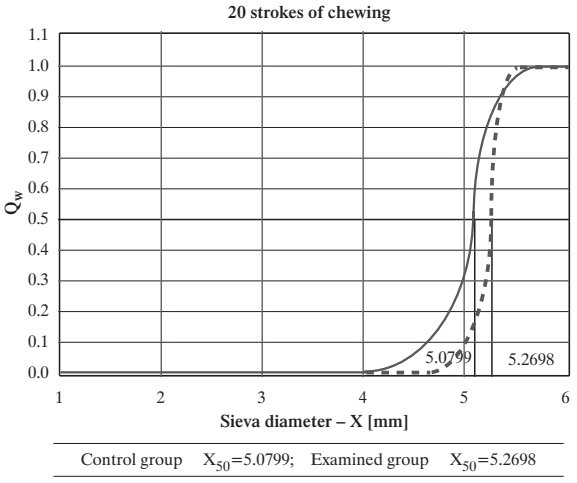
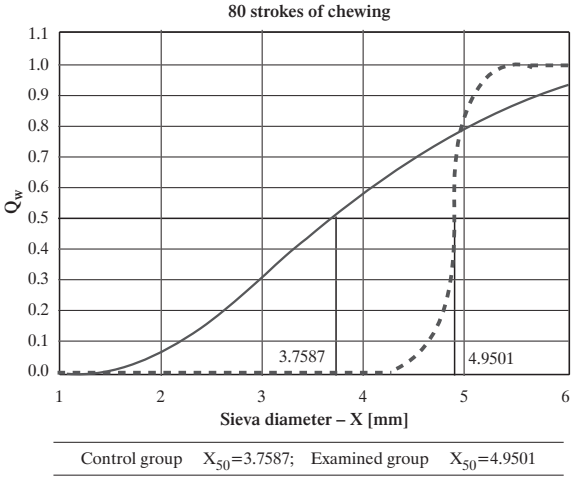


Figure 2. **The value of X_{50} for 80 cycles of chewing estimated in Optosil test**



Analyzing this data we can observe that all the differences are statistically significant.

The value of X_{50} for 20 and 80 cycles of chewing estimated in Optosil test as the measure of the ability of crushing food is presented in the *Tab. 3* and the *Fig. 1* and *2*. We have recorded the statistical significance between both groups only for 80 cycles of chewing.

It has also been found that there is a positive correlation between the force of pressure (r=0.6588, p=0.000), the chewing platform area (r=0.8138, p=0.000) and the number of functional dental units. The negative correlation has been recorded between the chewing platform area (r=-0.4983, p=0.002), the number of functional dental units (r=-0.6308, p=0.000) and the ability of comminution of solids for 80 cycles of chewing (*Tab. 4*).

Discussion

We formed the examined group selecting people whose occlusion had never been rehabilitated because prosthetic appliances could affect the masticatory patterns. Our patients had lack of teeth above 50%. As it was suggested the lack of teeth below 50% could be well compensated by existing in mouth teeth [15]. It was established that people with reduced number of posterior teeth report their chewing ability to be satisfactory as long as 20 well distributed teeth are present (3-4 occluding pairs preferably in a symmetrical position) [16,17]. One pair of occluding molars provides sufficient chewing ability [16]. Our patients from the examined group had evident problems with crashing food because of extremely shortened dental arches. A significant influence of the number of occlusal units on the masticatory performance was observed also by different investigators [18].

To establish the masticatory efficiency we chose the test based on chewing artificial food – silicon-rubber mass (Optosil). This test allowed the quantitative assessment of artificial food particles comminution as a function of the number of chewing strokes using standardised sieving method [19-21]. Therefore, we used just this test for the assessment of masticatory efficiency in our patients with suspected masticatory deficiency caused by

advanced teeth loss in comparison to the control group with good natural dental status.

As the result of lack of postcanine functional dental units the chewing platform area was considerably reduced. Moreover, the force of biting was reduced too. It was found during the estimation of occlusal central position in T-Scan II System registration. The necessary masticatory forces to prepare food in the mouth for digestion are 6 kg for each tooth during mastication, and the degree of force may vary according to the physical features of food [22]. The maximal forces which were determined using gnathodynamometer were established as over 90 kg in back segments and over 60 kg in the front segment but it was also recorded that there was a certain difference between the intraoral forces during mastication and measured maximum intraoral forces [23]. Evident diminishing of chewing platform area and force of crushing hard particles as the result of lost of teeth are caused the impairment of chewing ability.

It was suggested that impossibility of comminution of hard food was improved by prolongation of chewing [24-26]. Although, the mechanism of compensation the deficiency of chewing was not became clear but on the base of our investigation it was evident that short chewing of food did not cause the essential difference in crushing particles in the examined group independently of the state of dentition. Only prolongation of chewing improved the comminution of hard particles [24].

Our results showed that there was a close relationship between the number of teeth, the force of preassure on the indicator, the chewing platform area of teeth which takes part in mastication and the ability of comminution of food for 80 cycles of chewing by means during long chewing. Comparative results, carried out in Netherlands, recorded that chewing performance as measured with chewing tests, declined lineary with decrease of chewing platform area and extreme shortened dental arches comprising 0-2 occluding premolars resulted in severe impairment of chewing ability [16].

Conclusion

The severe reduction of the number of functional dental units is caused of the impairment of chewing ability but prolongation of mastication could improve the comminution of hard food.

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Assessment of the state of dentition and oral hygiene in 16-25-year-old young people with mild and moderate mental disability

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Abstract

Purpose: The purpose of the research is to assess the state of dentition and oral hygiene in 16-25-year-old young people with mild and moderate mental disability in comparison with a control group of healthy young people at the same age.

Material and methods: The research was carried out in a special School and Tutelary Centre in Lublin. A group of 144 young people aged 16-25 with mild and moderate mental disability (group I) among them 75 girls and 69 boys participated in the research. A group of 50 healthy young people aged 16-25 (group II) among them 24 girls and 26 boys was a control group. Determined: frequency of dental caries, DMF number, dental caries treatment index (DTI), oral hygiene index (OHI), percentage of traumatic injuries of teeth, percentage of sealed teeth.

Results: The frequency of dental caries in both groups was 100%. The average DMF was 11.96 (group I) and in the control group II: 6.58. The largest number of teeth with active caries – 8.21 teeth with caries per person was found in group I, but 2.72 in group II. Dental caries treatment index (DTI) was 0.24 in group I and 0.59 in the control group II.

Oral hygiene index OHI in group I was 1.78, in group II this index was 0.34, 0.29 in girls and 0.38 in boys.

Conclusions:

1. The state of dentition in 16-25-year-old young people with mild and moderate mental disability is unsatisfactory.
2. Higher values of OHI index were in group I.
3. The obtained results of the state of dentition and oral hygiene in the group of young people with mental disability are at the same level both in the girls and boys.
4. The above mentioned results suggest the need for special dental care for young people with mild and moderate mental disability.

Key words: state of dentition, oral hygiene, mild and moderate mental disability.

Introduction

According to the Health World Organization a disabled person is a person who for a longer time was excluded from full participation in the normal activity of their age group.

WHO distinguishes 5 groups of the disabled people:

Group I: the blind and visually impaired people

Group II: the deaf and poorly hearing people

Group III: people with a reduced intelligence quotient (IQ):

a. mild mental disability //=67-52

b. deeper mental disability

moderate //=51-36

considerable //=35-20

c. severe mental disability //=19-0

Group IV: socially non-adapted people

Group V: disabled children due to physical impairment and chronic diseases and thus disabled in a smaller or greater degree.

A constantly increasing number of the disabled in the contemporary world is worrying. This phenomenon is connected with the development of our civilization which causes on the one hand the improvement of life but on the other hand it impairs the inner (mental) balance of the man (acc. to Dubois). The simultaneous progress in medicine causes the decrease of natural selection. It manifests itself among others by the occurrence of mental diseases.

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***Table 1*. State of dentition and oral hygiene in examined persons with mental disability (group I) and in the control group (group II)**

	Caries frequency	DMF number	D	M	F	Treatment Index DTI	Percentage of person with teeth injuries (%)	Percentage of person with sealed teeth (%)	OHI
Investigated group (group I)	100%	11.96	8.21	1.07	2.68	0.24	4.13	4.16	1.78
Control group (group II)	100%	6.58	2.72	0.00	3.86	0.59	4.0	12.0	0.34

***Table 2*. State of dentition and oral hygiene in examined persons with mental disability considering sex (group I)**

Gender	Frequency	DMF	D	M	F	DTI	OHI
Girls	100%	10.89	8.30	0.89	1.70	0.17	1.57
Boys	100%	12.53	8.10	1.27	3.16	0.29	1.97

***Table 3*. State of dentition and oral hygiene in the control group considering sex (group II)**

Gender	Frequency	DMF	D	M	F	DTI	OHI
Girls	100%	6.33	3.00	0.00	3.33	0.52	0.29
Boys	100%	6.80	2.46	0.00	4.34	0.64	0.38

Mental disability is the functioning of the intellect at the lower level than average. Mild disability occurs when an IQ is within 52-67. Such disabled people do not differ in appearance from their peers. But with a closer observation disorders in the motor activity, lack of precise movements and a deviation in maturation and learning are seen. Moderate disability means a lower level in the functioning of the intellect within 36-51 manifesting itself in the reduction of social maturity [1-3].

Described mental limitations are a great obstacle in the contact with a patient in the dental surgery. They make it impossible to take a detailed case history and to carry out a planned dental treatment.

Purpose of the research

The purpose of the research is to assess the state of dentition and oral hygiene in 16-25-year-old young people with mild and moderate mental disability in comparison with a control group of healthy young people at the same age.

Material and methods

The research was carried out in a special School and Tutelary Centre in Lublin. A group of 144 young people aged 16-25 with mild and moderate mental disability (group I) among them 75 girls and 69 boys participated in the research. A group of 50 healthy young people aged 16-25 (group II), among them 24 girls and 26 boys living in a boarding school in Lublin was a control group. Clinical examinations were carried out in a dental surgery in the artificial light with the use of a diagnostic kit (a dental mirror and a probe).

The states of the following were taken into consideration during the examination:

- hard tissues of the teeth
- oral hygiene considering bleeding from gingival pockets

On the basis of clinical examination the following data were determined:

- frequency of dental caries
- DMF number
- dental caries treatment index (DTI)
- oral hygiene index (OHI)
- percentage of traumatic injuries of teeth
- percentage of sealed teeth.

The results were statistically collated using Mann-Whitney test in Statistica 6.0 programme, group I and the control group II were compared considering sex of the examined people.

Results

The frequency of dental caries in both groups was 100%. The average DMF number in mentally disabled people (group I) was 11.96 and in the control group II: 6.58. The largest number of teeth with active caries – 8.21 teeth with caries per person was found in group I, but 2.72 in group II. The number of extracted teeth due to dental caries was on average 1.07 in group I and 0.00 in group II. The number of fillings was on average 2.68 in group I and 3.86 in group II. DMF number was 10.89 in girls and 12.53 in boys in group I. In group II this number was 6.33 in girls and 6.80 in boys.

Dental caries treatment index (DTI) was 0.24 in group I and 0,59 in the control group II.

The percentage of injuries was 4.13% in group I and 4.0% in group II.

The percentage of persons with sealed teeth was 4.16% in group I and 12% in group II.

Oral hygiene index OHI in group I was 1.78, 1.57 in girls and 1.97 in boys.

In group II this index was 0.34, 0.29 in girls and 0.38 in boys.

The above mentioned research results are collated in *Tab. 1-5* (*Tab. 1*).

Analysing the obtained results in the group of young people with mild and moderate mental disability and comparing them with the results obtained in the control group significantly statistical dependences of the state of dentition and hygiene index OHI in shown both groups. The level of statistical significance was <0.05.

The obtained dependences are depicted in *Fig. 1-2*.

Considerably significant statistical differences were stated in the assessment of the level of oral hygiene in pupils from School and Tutelary Centre (group I) in comparison with healthy young people from the boarding school (group II).

Table 4. **Statistical analysis of investigated parameters determining the state of dentition and oral hygiene in both groups**

GROUP I-Young people with mental disability	N valid	Average	Median	Statistical deviation
OHI number	144	1.78472	2.00000	0.765636
D number	144	8.20833	8.00000	4.235836
M number	144	1.07639	0.00000	1.718187
F number	144	2.68056	2.00000	2.669833
DMF number	144	11.96528	11.00000	4.111068
Group II-Healthly young people	**N valid**	**Average**	**Median**	**Statistical deviation**
OHI number	50	0.34000	0.00000	0.478518
D number	50	2.72000	3.00000	1.829910
M number	50	0.00000	0.00000	0.000000
F number	50	3.86000	4.00000	2.878563
DMF number	50	6.58000	6.50000	2.556225

Table 5. **Value differences of investigated parameters determining the state of dentition and oral hygiene between group I (young people from School and Tutelary Centre) and the control group II (healthy young people)**

	Sum. rang	Sum. rang	U	Z	Level p	Z	level p	N valid	N valid
Index OHI	1817.000	17098.00	542.000	-8.94014	0.000000	-9.33515	0.000000	50	144
D number	2150.500	16764.50	875.500	-7.96515	0.000000	-7.99032	0.000000	50	144
M number	3225.000	15690.00	1950.000	-4.82382	0.000001	-5.73102	0.000000	50	144
F number	5763.500	13151.50	2711.500	2.59755	0.009389	2.63312	0.008461	50	144
DMF number	2115.000	16800.00	840.000	-8.06893	0.000000	-8.09489	0.000000	50	144

Figure 1. **Values of DMF number**

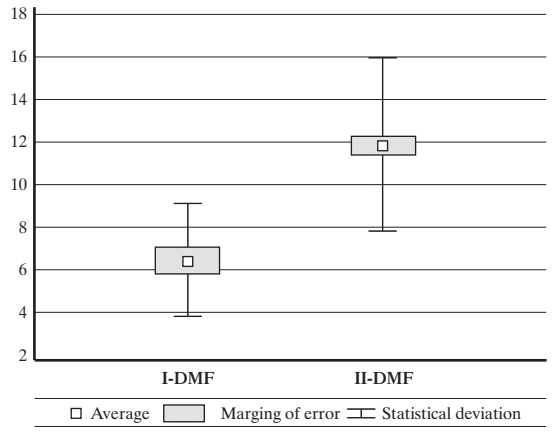
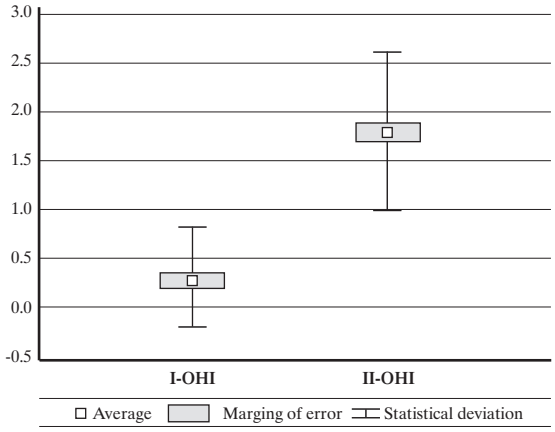


Figure 2. **Value of index of oral hygiene OHI**



OHI index determining the state of oral hygiene has considerably higher values in group I than in the control group II the level of statistical significance was <0.05.

That is confirmed by considerably more frequent occurrence of bleeding from gingival pockets in pupils from group I (young people with mild and moderate disability) than in the control group II (healthy young people).

Discussion

The state of dentition in 26-25-year-old young people with mild and moderate mental disability is unsatisfactory, which is manifested by 100% frequency of dental caries and a greater number of teeth with active caries.

Mielnik-Błaszczak et al. obtained similar results of the state of dentition in children and young people with special needs and of their assessment of permanent teeth health in children and young people from School and Tutelary Centre in Krosno [4,5].

Borysewicz-Lewicka at al. assessing the state of dentition in pupils from a special needs school also found a high frequency of caries which is connected with unsatisfactory primary dental care of children with special needs [6].

Similar conclusions are drawn by authors of Scandinavian publications concerning research carried out in specialist centres for children and young people with a various degree of mental disability. They clearly show the need for prophylactic activities among mentally disabled children and young people and for the creation of dispensary groups with intensified dental care [7-9].

Andruszkiewicz-Sałek proposes a model of dental care for patients with cerebral palsy which would consider early prophylaxis of dental caries and periodontal disease [10].

The results of our own research show that health awareness and oral cavity hygienic habits are lower in mentally disabled young people than in the healthy ones. It shows the need to work out a similar model of prophylactic and therapeutic activities for those under the charge of School and Tutelary Centres.

Conclusions

1. The state of dentition in 16-25-year-old young people with mild and moderate mental disability is unsatisfactory. It is manifested by 100% frequency of dental caries and a higher number of teeth with active caries compared with the group of healthy young people.

2. Higher values of OHI index and a more frequent bleeding from gingival pockets in young people from School and Tutelary Centre indicate a worse state of oral hygiene compared with the control group of healthy young people.

3. The obtained results of the state of dentition and oral hygiene in the group of young people with mental disability are at the same level both in the girls and boys.

4. The above mentioned results suggest the need for special dental care for young people with mild and moderate disability and to work out a special prophylactic and therapeutic programme for this group of patients.

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Assessment of periodontal status following the alignment of impacted permanent maxillary canine teeth

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Abstract

Purpose: The aim of the study was to assess the effect of orthodontic movement of the impacted canines after surgical exposure and alignment on the periodontal status of the transpositioned and adjacent teeth as well as to compare certain parameters with those of spontaneously erupted teeth.

Material and methods: Twenty-four patients (mean age 18.4±3.66) with unilaterally impacted 24 canines were enrolled in the study. The following parameters were assessed: pocket depth (PD), clinical attachment level (CAL), platelet index (PI) of Silness and Löe, and modified sulcus bleeding index (SBI). Optic density of the alveolar bone along the root surface of the aligned canine was analysed based on digital radiological images made with the right angle technique. Control group consisted of spontaneously erupted teeth.

Results: In comparison to the control group, in the orthodonticaly treated group PD was found to increase on the mesial buccal and palatal surfaces of the first premolar ($p<0.003$, $p<0.04$), on the treated side; on the distal buccal ($p<0.01$), mesial buccal ($p<0.0005$), mesial palatal ($p<0.02$) and distal palatal surfaces of the canine ($p<0.02$); and on the distal buccal ($p<0.04$) and distal palatal surfaces of the lateral incisor ($p<0.048$). CAL was statistically significant on the mesio-buccal and mesio-palatal surfaces of the aligned canine ($p<0.02$). PI was statistically insignificant, while SBI values at the aligned tooth were statistically significant ($p<0.0004$). Positive correlation was found between treatment duration and distance to the occlusal plane (d) expressed by the correlation coefficient $r=0.49$ ($p<0.02$).

No relationship was observed between bone density within the canine alignment zone and the control, and there was no link between the method of treatment and periodontal status, either.

Conclusions: The alignment of the impacted permanent maxillary canines poses a risk of periodontal deterioration. Patients subjected to surgical-orthodontic treatment require periodic periodontal follow-ups.

Key words: impacted permanent maxillary canines, periodontal status, therapeutic methods.

Introduction

The impacted teeth located in the frontal segment of the maxilla frequently need to be surgically exposed and due to the application of extrusive forces erupt towards the occlusal plane [1,2]. The impaction of permanent maxillary canines is the most common, with a rate that varies between 1% and 3% [3-5]. Bishara et al. [3], summing up Moyers' theory, have stated that canine impaction may have primary causes (deciduous root resorption rate, injury to deciduous tooth bud, eruption disorders, arch space deficiency, bud rotation, preterm closure of the root apex, eruption of the canine close to a fissure that adjoins cleft palate) as well as secondary causes (abnormal muscle tone, pyretic diseases, hormonal disturbances, vitamin D deficiency). According to some researchers, genetic disorders can be responsible for malalignment of the canine, while others provide evidence confirming the guidance canine eruption theory based on the leading role of the lateral incisor root [3,6].

Orthodontic management in the case of impacted maxillary canines requires carefully planned interdisciplinary co-operation [2]. The canines are exposed surgically and fixed orthodontic appliances bonded to the exposed teeth are used

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Table 1. **Impaction zones with regard to patients' age, treatment duration and distance to the occlusal plane of the impacted canines (d)**

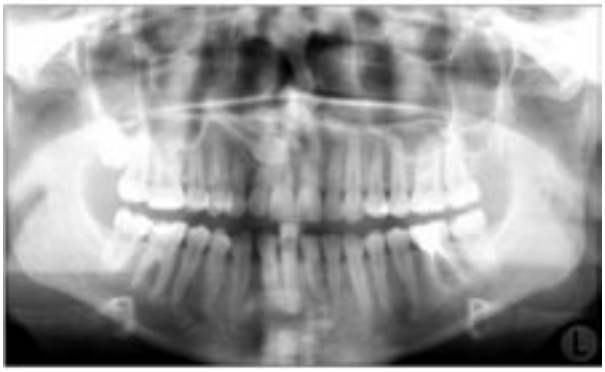
Impaction zones		Patients age	Treatment duration	d
I	*n*	2	2	2
	Mean	18.50	20.50	13.50
	SD	3.68	3.54	0.71
II	*n*	5	5	5
	Mean	19.56	21.20	17.20
	SD	5.27	3.11	7.33
III	*n*	5	5	5
	Mean	17.94	19.60	12.20
	SD	3.80	4.28	1.79
IV	*n*	7	7	7
	Mean	16.67	24.29	15.57
	SD	1.84	1.60	3.69
V	*n*	5	5	5
	Mean	20.06	23.40	17.20
	SD	3.95	8.88	3.96
Total	*n*	24	24	24
	Mean	18.40	22.17	15.38
	SD	3.66	4.83	4.49

n – number of the canines; * – t Student test

for their alignment [3,7]. Then traction is applied in order to move the impacted tooth in the desired direction. With the optimum force applied, ranging from 10 to 100 g [6], the teeth move in the alveolar process. They are hold by the surrounding collagen structure, i.e. periodontal ligaments (PDL), containing non-differentiated mesenchymal and daughter cells: fibroblasts and osteoblasts, blood vessels, nerve endings and periodontal fluid. The orthodontic shift of the teeth is a response of biological reactions inside PDL and alveolar bone to the external force released by orthodontic appliances. Since surgical exposure of the palatally impacted canine reveals its lingual surface, it is there where orthodontic elements can be fixed. When canines are vestibularly impacted, brackets are placed directly on the labial surface. In order to apply orthodontic force to the tooth by means of orthodontic brackets, the following procedures should be instituted: firstly – after surgical exposure, vertically directed orthodontic force should be applied to allow movement of the tooth and shift from the roots of the adjacent teeth; secondly – exposure of the canine should take place after initial nivelization of the arch, with the possibility of stretching the traction to the rigid marginal arch. Such management delimits the occurrence of force which has undesirable effect on the periodontal tissues and minimizes the risk of root resorption. However, it should be remembered that after the application of orthodontic force the impacted teeth exhibit relatively slow movement in the bone and prolonged treatment duration may have an unfavourable effect on periodontal status.

Treatment involves canine exposure and alignment, with proper occlusion, healthy gums, right length of the crown and ideal height of the alveolar process maintained [8,9]. This, however, requires co-operation between oral surgeon, orthodontist and periodontist.

Figure 1. **Panoramic radiogram of a 16-year-old female patient with impacted tooth 13 in zone V**



The aim of the study was to assess the effect of orthodontic movement of the impacted canines after surgical exposure and alignment on the periodontal status of the transpositioned and adjacent teeth as well as to compare certain parameters with those of the homonymous spontaneously erupted teeth.

Material and methods

Twenty-four patients (19 girls and 5 boys) with 24 unilaterally impacted canines were enrolled in the study. The mean age of patients was 18.4±3.66. The impacted teeth were localized on the basis of clinical examination and panoramic radiogram using the method modified from Ericson and Kurol [10], (of zone I-V, distance to occlusal plane – d). When the impacted canine was recognized, fixed appliance (slot .018) was attached in order to align the teeth before surgical procedure. Following the nivelization phase, a rectangular steel arch 17x25 was inserted in the brackets with an additional passively ligatured spring and accessory steel arch (.016) with a "ballista" loop according to the Jacoby method [11]. Next, in local anesthesia, the tooth was surgically exposed (the choice of the method depended on the impaction zone and distance to the occlusal plane d – *Tab. 1*, *Fig. 1*); its range was consulted with the orthodontist. After haemostasis, an orthodontic bracket was attached, a flap was sewn or the tooth was left uncovered with an inserted golden chain, and it was activated by connecting it to the "ballista" loop with elastic thread (*Fig. 2*).

The clinical examination was performed after tooth alignment (*Fig. 3*, *4A-B*). A periodontal probe was used for examinations. The following parameters were assessed: pocket depth (PD in mm), plaque index (PI) according to Silness and Löe scale 0-3, modified sulcus bleeding index (SBI) and clinical attachment level (CAL in mm).

In radiological examination, digital radiography method with the right angle technique was employed to take pictures on the side of the aligned tooth and on the side of the spontaneously erupted canine. Then, alveolar bone density along the root surface of the translocated canine was analysed, by measuring point by point from the distal side of the lateral incisor, using

Figure 2. **Radiogram presents initial activation of a ballista loop in the extrusion phase in tooth 13**

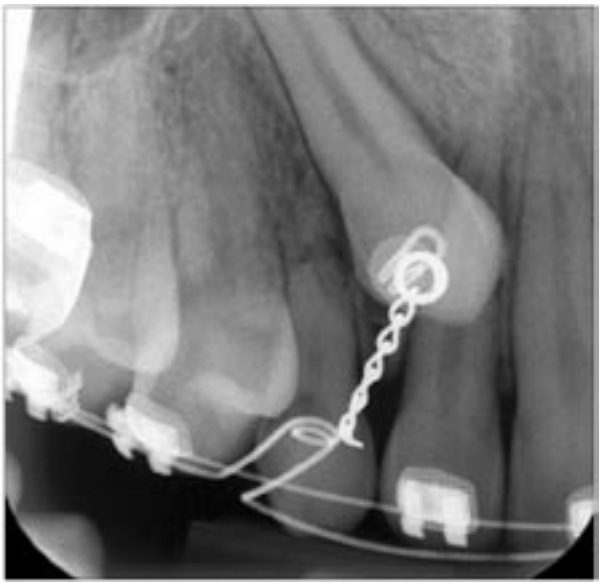
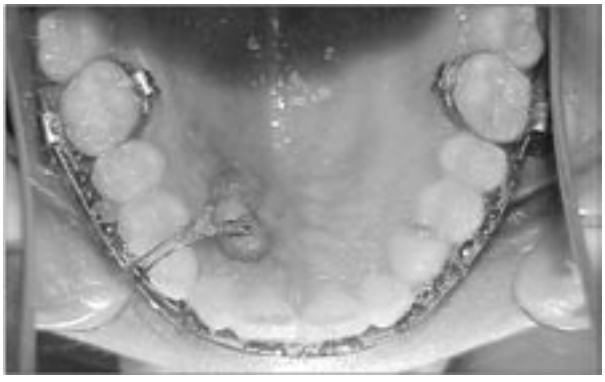


Figure 3. **Tooth 13 after two-month ballista loop activation**



a computer programme for measurement of optical density of bones. The control group consisted of spontaneously erupted teeth in the enrolled patients.

Statistical analysis

Results were subjected to statistical analysis, using t-Student test, non-parametric Wilcoxon test and Pearson correlation coefficient for paired variables.

Ethics

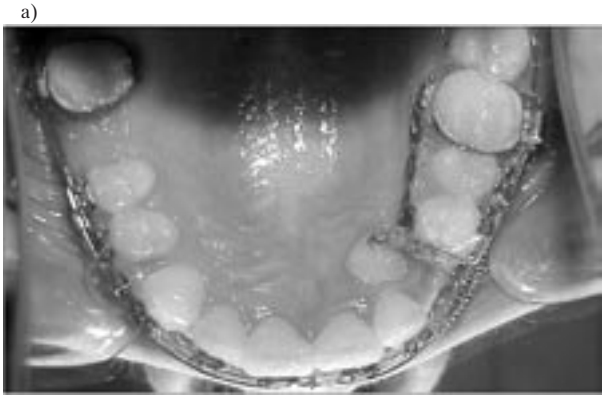
The Ethics Committee of Medical University of Białystok accepted the study.

Results

Tab. 1 presents distribution of the impacted canines with reference to the impaction zones, age, treatment duration and distance to the occlusal surface. Most canines were impacted in zone IV (7/24), then in II, III and IV (5 in each), with 2 canines

Figure 4 A-B. **Successive stages of left canine alignment in an 18-year-old female patient**

a)



b)

Table 2. **Distribution of surgical methods with reference to canine impaction zones**

Impaction zones	Surgical methods		Total
	open	close	
I	1	1	2
II	0	5	5
III	1	4	5
IV	1	6	7
V	1	4	5
Total	4	20	24

n – number of the canines; * – t Student test

in zone I. The impacted canines in zone IV were found in the youngest patients (mean age 16.67 years), in zone V in the oldest (mean age 20.06 years). The longest distance to the occlusal surface was in zones II and IV (17.20 mm in each), which was connected with the longest time of treatment. The canines impacted in zone V were being aligned for the mean period of 23.4 months, in zone III for 19.6 months. *Tab. 2* presents the surgical methods of the exposure of 24 impacted canines, of which 20 required the closed technique.

PD measurements assessed with the Wilcoxon non-parametric test for paired variables showed an increase on the mesial buccal and mesial palatal surfaces of the first premolar ($p<0.003$, $p<0.04$), on the side of the aligned tooth. Canine PD values were statistically significant for the distal buccal surface

Table 3. **Clinical assessment of probing depth (PD) and attachment level (CAL)**

Tooth surface	PD		CAL	
	Mean/SD	p value	Mean/SD	p value
First premolar				
mesio-buccal surface of the treated tooth	2.23±0.93	0.003*	1.53±1.14	0.07
mesio-buccal surface of the controlled tooth	1.63±0.89		1.13±0.84	
mesio-palatal surface of the treated tooth	1.19±0.89	0.04*	1.15±0.93	0.14
mesio-palatal surface of the controlled tooth	1.00±0.71		1.00±0.71	
Canine				
distal buccal surface of the treated tooth	2.48±1.20	0.01*	1.35±1.08	0.48
distal buccal surface of the controlled tooth	1.77±0.86		1.15±0.94	
mesio-buccal surface of the treated tooth	2.63±0.96	0.0005*	1.58±0.96	0.02*
mesio-buccal surface of the controlled tooth	1.56±0.84		1.15±0.99	
mesio-palatal surface of the treated tooth	1.46±1.02	0.02*	1.58±0.96	0.02*
mesio-palatal surface of the controlled tooth	1.08±0.87		1.15±0.99	
distal palatal surface of the treated tooth	1.52±0.90	0.02*	1.27±0.92	0.13
distal palatal surface of the controlled tooth	1.13±0.98		1.08±0.96	
Lateral incisor				
distal buccal surface of the treated tooth	1.96±0.76	0.04*	1.23±0.90	0.19
distal buccal surface of the controlled tooth	1.85±2.06		0.98±0.91	
distal palatal surface of the treated tooth	1.17±0.80	0.048*	0.92±0.82	0.60
distal palatal surface of the controlled tooth	0.90±0.66		0.88±0.73	

* – Wilcoxon non-parametric test for paired variables; Table contains statistically significant results of the test (p*)

Table 4. **Comparison of mean values of modified bleeding index SBI in the groups**

Modified bleeding index SBI	Mean/ SD	p value
SBI treated group	32.25±24.97	0.0004
SBI control group	19.42±20.79	

* – t Student test

Table 5. **Analysis of correlation between age, treatment duration and distance to the occlusal plane**

	r	p
Age× treatment duration	0.25	0.24
Age × d	0.14	0.51
Treatment duration × d	0.49	0.02

r – Pearson correlation coefficient for paired variables; p – statistically significance of the correlation coefficient given statistically significant positive correlation between treatment duration and distance

(0.01), mesial buccal surface (p<0.0005), mesial palatal surface (p<0.02) and distal palatal surface (p<0.02). Compared to the control group, lateral incisor PD in the study group was statistically significant for the distal buccal (p<0.04) and distal palatal surface (p<0.048).

CAL was statistically significant on the mesio-buccal and mesio-palatal surfaces of the aligned canine as compared to the control (p<0.02) (*Tab. 3*). In the study group, PI values were not statistically significant, while SBI was statistically significant at the aligned tooth compared to the control group (p<0.0004) (*Tab. 4*).

Positive correlation was found between treatment duration and distance to the occlusal plane (d) expressed by the correlation coefficient r=0.49 (p<0.02) (*Tab. 5*). No relationship was observed between bone density in the alignment zone as compared to the control. No relationship was found between the method of treatment and periodontal status.

Discussion

Surgical exposure of impacted maxillary canines and their alignment by means of fixed orthodontic appliances is a common method of treatment. Periodontists believe that orthodontic treatment by means of fixed appliances may cause chronic inflammation of the periodontal margin [9,12], and that the changes induced may play a major role in aetiopathogenesis of periodontal diseases at a later age. Among the factors of pathogenic diseases dental abnormalities are mentioned [13].

The study results confirm that the process of impacted tooth alignment is accompanied by changes in the structure of the periodontal tissue. This is perhaps associated with a long-lasting process of forced orthodontic eruption of the impacted canines, especially when the regained tooth has a difficult tortuous distance to the occlusal plane. Lack of proper oral hygiene during fixed appliance therapy leads to dental plaque accumulation that may trigger the inflammatory process [12,14].

Differences in the depth of gingival crevices at the aligned canines were increased on the mesial and distal sides of the buccal and palatal surfaces. So were those at the adjacent teeth, i.e. in the first premolars – mesio-buccally and mesio-palatally, and in the lateral incisors – disto-buccally and palatally. However, the crevice depth oscillated from 1.17±0.80 to 2.65±0.96 mm. These results are consistent with the data reported by other authors [13,15]. A detailed analysis of the periodontal tissues

after surgical exposure of maxillary canines has been conducted by Gaulis and Joho [16], who noted that tissue removal, particularly in the case of vestibular impaction, can have an unfavourable effect on mucosa and gingiva. In such cases, flap should be shifted to the crown. Vanarsdall and Corn [17] and Vermette and Kokich [18] described the technique that helps to avoid soft tissue recession and bone loss during treatment of vestibularly misaligned unerupted teeth. Radical surgical exposure of the orthodontically treated impacted maxillary canines results in greater pocket depth and bone loss [19,20]. Surgically exposed teeth erupt when force released by active orthodontic elements is applied [1,8,11]. At first, the force vector is vertically oriented for extrusion, and then the force works in the buccal direction. The canines moved from the impaction zone need derotation and additional torque, especially when the teeth show palatal displacement [13]. In the present study, the closed technique was the predominant one (20/24). Although surgical canine exposure with flap and its sewing were done with great care and precision, CAL was found to be lowered on the mesio-buccal and mesio-palatal surfaces of the impacted canine. The loss of attachment may be due to the orthodontic procedures or/and it may be caused by injury during toothbrushing [14]. In a group of patients with no hygienic regime instituted, Suomi et al. [21] demonstrated the loss of attachment of 0.10 mm per year in a 3-year observation. A similar view has been presented by Zachrisson [9], who tried to explain the effect of fixed appliances on the periodontal status in a group of 16-year-old teenagers in comparison to orthodontically untreated patients. His study revealed a 0.4 mm loss of attachment in canines in the treated group and 0.1 mm in the control group, with 2 mm PD found in both groups. Chaushu et al. [22], who examined 11 canines aligned in the open method procedure, described a considerable loss of CAL of the impacted teeth, which was associated with gingival recession without substantial deepening of the gum crevices labially but with their slight deepening and bone loss mesially. Recession, however, was not observed at the aligned canines, which can be explained by the proper supervision of the treatment, with the minimum palatal leaning of the root and crown shift towards the vestibule in the exposed canine.

The PI values of the teeth adjacent to the impacted and spontaneously erupted teeth were not statistically significant, which is consistent with the data reported by Kohavi et al. [13] and Zachrisson and Alnaes [23]. This is certainly associated with frequent visits (in the extrusion phase at least twice a month) and intensified hygienic procedures. Wisth et al. [20] also support this opinion. However, in the open technique, dental plaque may accumulate when the exposed canine is spared and thus not brushed properly [23]. In the study group of patients with impacted canines, SBI measurement at the aligned tooth was statistically significant in comparison to the control group. This may indicate an inflammatory process taking place in the gingival crevices.

From the biomechanical point of view, when there is not sufficient room for the canine, light vertical force should be applied locally. The use of elastic chains or threads to obtain single eruption force from the rigid basic arch should produce slight force due to a substantial bend resulting from loading and rapid loss of force provided by elastic elements.

Alignment of a larger number of teeth causes that side-effects can spread over the enlarged surface area of the roots and thus become attenuated. In all the cases of the aligned teeth, slight force of 60 g released by the activated “ballista” loop was applied. The length of the loop arm should equal the distance measured from the retained tooth to the occlusal plane, which produces enough force for mild shift of the canine in the bone tissue tunnel [11]. The use of the “ballista” loop lasts till the canine cusp appears in the gingiva zone. Then a bracket is properly attached and treatment is continued on the rectangular arches to even the tooth axis and achieve the appropriate torque. Determination of the exposed tooth gradient is an extremely important clue for the orthodontist, as it is its long axis that determines the direction of traction. In the current study, all the exposed teeth had orthodontic golden brackets stuck to them, with a chain which due to its plasticity is the least traumatizing and does not injure the soft tissues. Time of impacted tooth alignment positively correlated with the distance to the occlusal plane – more deeply retained canines required longer therapy. These data are in agreement with those reported by Stewart et al. [24]. Lack of changes in bone density after alignment of exposed and transpositioned canines may indicate that movements of the teeth induced by orthodontic force are comparable to the changes that accompany their physiological eruption.

The retained and aligned teeth subjected to surgical exposure require constant monitoring throughout the orthodontic treatment followed by periodontal care after termination of the active phase. Improvement in periodontal status is associated with regular hygiene, especially in the final stage of canine alignment.

Conclusions

1. Alignment of impacted maxillary canines is associated with the risk of periodontal status deterioration.
2. Patients treated with the surgical-orthodontic method require periodical periodontal follow-ups.

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Oral cavity status and IgE level in orthodontic patients

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Abstract

Purpose: Considering nickel release from fixed orthodontic appliances, determination of the relationship between the clinical status of the mouth, IgE level and treatment duration in orthodontic patients seems to be advisable.

Material and methods: Twenty-one patients with symptoms of nickel hypersensitivity observed during treatment with fixed orthodontic appliances were separated from a group of 50 subjects, aged 11-33 years, undergoing orthodontic treatment for malocclusion. The patients were divided into two subgroups PgA and PgB.

Results: The mean IgE level in PgA was 39.20 IU/ml and in PgB 210.61 IU/ml. In PgA, the majority of patients were wearing ear-rings (8/10), but not in PgB (4/11). The mean treatment duration in PgA was 21.3±4.83 months, while in PgB 14.4±2.84. There were no statistically significant differences in the symptoms indicating stomatitis between the groups of patients subjected to treatment with intra-oral appliances.

Conclusions: The immunologic profile of the patient plays a key role in the choice of the type of appliance used to treat abnormalities of the masticatory organ. Determination of IgE is necessary in the case of allergy-positive history.

Key words: nickel allergy, immunologic profile, orthodontic treatment.

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Introduction

The common use of fixed appliances for the treatment of occlusal and dental abnormalities requires a guideline of procedure standards to follow in case of nickel intolerance. Nickel is a component of many orthodontic materials, including brackets, rings and arches. Kanerva et al. [1] and Kerosuo and Kanerva [2] described the effect of nickel released from the steel of fixed appliances on contact dermatitis as type IV of delayed hypersensitivity reaction. The mean nickel content ranges from 8 to 30% [3]. It is a very strong allergen and its presence in the oral cavity can induce a cascade reaction manifested in reddening, softening and hyperplasia of the gums, bleeding, angular cheilititis or dermal exanthema of varied intensity [4,5]. Clinical symptoms of nickel allergy may resemble periodontitis and mucosal erosions can be seen as the effect of friction between the superficial epithelial layer and the appliance. Literature reports concerning the amount of nickel released in the course of treatment are contradictory. In the study conducted by Agaoglu et al. [3] with the saliva of 100 patients subjected to a 24-month-orthodontic treatment, the level of nickel increased in the first week up to 4.45ppb, reached the peak (11.53 ppb) after a year, but returned to the first-week values at the end of the second year. The results are inconsistent with the data reported by Kerosuo et al. [6], who found no statistically significant increase in the salivary nickel level in the first months of treatment. According to Carvalho [7], in the initial phase, allergy is confined to local inflammatory changes, while in a long-term exposure the patients exhibit inflammatory, hyperkeratic and ulcerative fields in the oral cavity [8].

Therefore, determination of the relationship between the clinical status of the mouth, IgE level and treatment duration in orthodontic patients seems advisable.

Table 1. **Profiles of orthodontic patients with regard to age, worn jewellery and the time of exposure to orthodontic appliance in IgE subgroups**

Clinical characterization	PgA (n=10)	PgB (n=11)	p value
Average age (range)	16.0 (13-29)	14.0 (11-33)	p>.05
Average IgE	39.20 IU/ml	210.61IU/ml	p<.05
Range IgE	11.50-90.00	115.0-472.0	
Jewellery	8	4	p>.05
Average exposure time to orthodontic appliance	21.3±4.83	14.4±2.84	
Range (month)	(3-48)	(1-28)	

Table 2. **Inflammatory symptoms in subgroups A and B**

Inflammatory	PgA n=10	PgB n=11	p value
Symptoms	IgE=39.20IU/ml	IgE=210.61IU/ml	
Stomatitis	2	2	>.05
Reddened gum	6	4	>.05
Bleeding gum	6	4	>.05
Gingival hyperplasia	7	5	>.05
Angular cheilititis	4	4	>.05
Atopy	0	2	>.05

Material and methods

Twenty-one patients with symptoms of nickel hypersensitivity observed during treatment with fixed orthodontic appliances were separated from a group of 50 subjects undergoing orthodontic treatment for malocclusion. Immunoglobulin E was determined in the patients and according to the findings two subgroups were distinguished: A (10 patients with IgE <100 IU/ml) and B (11 patients with IgE >100 IU/ml).

Ethics

The Ethics Committee of Medical University of Białystok accepted the study.

Results

Tab. 1 presents patients' distribution with regard to age, nickel-containing jewellery worn by patients and orthodontic treatment duration. PgA and PgB were comparable with regard to age. The mean IgE level in PgA was 39.20 IU/ml and in PgB 210.61 IU/ml. In PgA, the majority of patients were wearing earrings (8/10), but not in PgB (4/11). The mean time of treatment in PgA was 21.3±4.83 months, while in PgB 14.4±2.84. There were no statistically significant differences in the symptoms indicating stomatitis between the groups of patients subjected to treatment with intra-oral appliances (*Tab. 2*).

Discussion

The current study indicates that determination of IgE level is an extremely useful marker helping to assess whether the inflammatory changes observed during fixed appliance therapy are the result of hygiene neglect or are due to reaction to the released nickel [1,9] (*Fig. 1*). Gingivitis, bleeding, hyperplasia are associated with dental plaque, which in orthodontic patients has favourite conditions for accumulation. Lack of proper hygienic regime causes a slow but steadily progressing destructive effect on periodontium, promotes hard tissue demineralization and increases the risk of caries. On the other hand, the appliance itself is not a threat when appropriate level of hygiene is maintained. However, in cases when despite proper hygienic procedures gum hyperplasia and bleeding are still observed (*Fig. 2*), determination of IgE should be considered to check if the person is allergic to nickel [1,10]. According to literature data, the incidence of allergy to nickel has increased in the past 10 years in Europe and USA in the proportion of 1 man: 8 women [11]. Most frequently, allergy appears in women between 16 and 35 year of life [12].

Figure 1. **Angular cheilititis as the effect of nickel hypersensitivity**

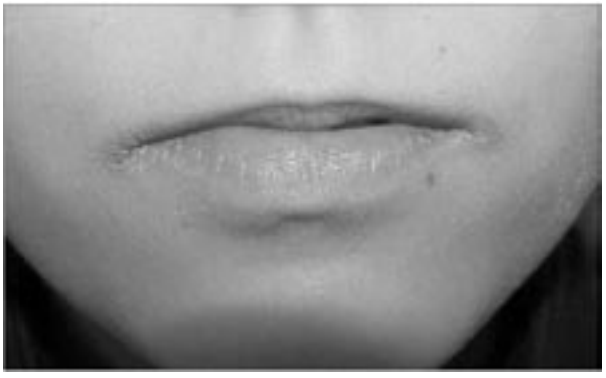


Figure 2. **Gingival hyperplasia caused by response to nickel**

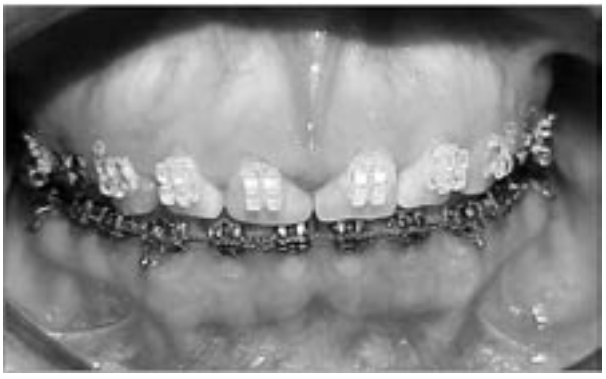
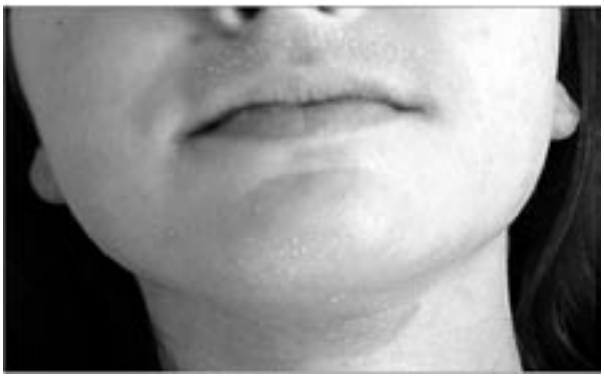
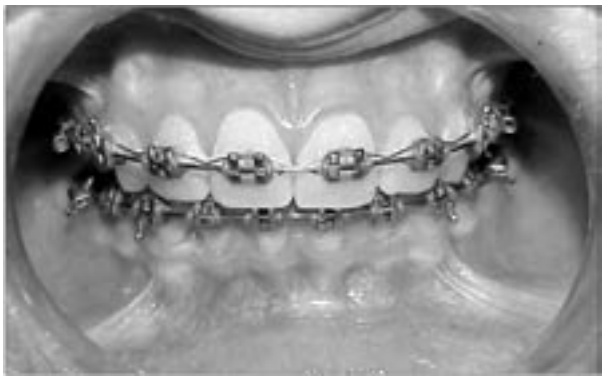


Figure 3A-B. **Marked exacerbation of atopy with simultaneously satisfactory periodontal status**





Nickel released to the oral cavity in the course of orthodontic treatment stimulates the immune process in the body. It has been estimated that the level of 30 ppm is likely to induce a cytotoxic response. Faccioni et al. [13] have demonstrated that nickel and cobalt impair DNA generation in oral mucosal cells. In normal processes, the cells have restorative capabilities, but when these are disturbed, the whole enzymatic process being a cell response to the operating cytotoxic compound is impaired. The time of treatment was nearly twice as short in PgB than in PgA, which confirms the opinion of Agaoglu et al. [3] that the greatest amount of nickel is released in patients with considerably elevated IgE. A fixed metal appliance that remains in the oral cavity for 24-48 months can emit a metal dose causing a cytotoxic effect [13]. According to Kocadereli et al. [14], the levels of metals that permeate to saliva, blood and urine during orthodontic treatment is below the accepted daily dose and does not cause any damage at the cellular level. On the other hand, the status of the oral cavity is also affected by biochemical processes that occur between oral hygiene preparations and metabolic products of dental plaque bacterial flora. Most PgA patients had been wearing nickel-containing ear-rings or other jewellery before insertion of the appliance. Nickel in ear-rings, watches, coins, after multiple contacts, may cause allergic reactions in some cases [15]. Lack of statistical significance in the occurrence of symptoms between the two subgroups allows the assumption that nickel released in very small amounts during orthodontic treatment causes the so-called tolerance [8,16,17]. However, it should be emphasized that atopy patients are particularly at risk, even when nickel doses are slight (*Fig. 3A-B*). Case history data are a valuable source of information about a patient's history of allergy. When hypersensitivity to nickel has been found, a change of appliance has to be considered. The new appliance should be made of the best quality anticorrosive materials, which do not induce any processes responsible for cell destruction.

Conclusions

The immunologic profile of patients plays a key role in the choice of the type of appliance used to treat abnormalities of the masticatory organ. Determination of IgE is necessary in the case of allergy-positive history.

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Recession occurrence in patients treated with fixed appliances – preliminary report

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Abstract

Purpose: The aim of the study was to evaluate the dependence of gingival recession, malocclusion and factors that may lead to recessions of the gingiva in patients applying for orthodontical treatment.

Material and methods: The study involved 52 randomly selected patients treated with fixed appliances due to occlusal irregularities and dental abnormalities. Data obtained from the examination and selected parameters from cephalometric analysis were placed in a chart including ANB skeletal class and Wits parameter, lower incisor position IMPA, dental abnormalities, extractions due to orthodontical indications and recession etiopathic factors.

Statistical analysis of obtained data was conducted using variance analysis. Statistically significant were assumed those calculations for which value of significance level $p \leq 0.05$.

Results: Recession was observed in 18 patients of the 52 examined. Skeletal I class was found in 11 patients (61.12%) and in 8 cases Wits parameter corresponded with skeletal class III (44.4%). Among the patients examined normal incisor inclination was observed in 6 patients (33.33%), whereas 12 cases revealed inclination irregularities (66.66%).

Clinical examination disclosed dental defects in 13 patients (72.22%) and in 12 cases recession etiopathic factors were recognized (66.66%). Statistically significant differences between periodontal biotype and gingival recession of 43, 31, 33 teeth were estimated.

Conclusions: Anatomical factors, malocclusion and dental irregularities were fund to be the main cause of the single and/or multiple recessions. Patients applying for orthodontic treatment due to occlusal abnormalities should remain under particular control in case of symptoms suggesting the incidence of recession, particularly when recessions are already present.

Key words: gingival recession, fixed appliances, etiopathogenesis.

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Introduction

Gingival recession according to McComb [1] defines a static state where we can observe a considerable dislocation of gum margin what as result leads to losing the width of attached gingiva and development of recession. For the patients suffering from gingival recession it invariably means hypersensitivity of dentin leading not only to paracervical defects of non-carietic origin but also to the root decay.

Etiopathogenesis of the recession is intricate and the generation of the abnormalities results from interaction of various factors which are often impossible to identify thus the assessment of their impacts remains as such [2,3]. According to Zachrisson et al. [4] the primary agent responsible for recession occurrence is improper teeth cleaning technique. The high level of oral cavity cleaning processes does not necessarily prevent recession. This situation applies mainly to patients, with no plaque records, who brush their teeth too often applying evidently too strong force on the toothbrush, giving rise to mechanical damage of paradontal area thus causing gingival recession [2,3,5-7]. The other noticeable factor is definite lack of oral hygiene, resulting in plaque inducing inflammatory reaction. Bacteria found in plaque and their metabolic products impair the connective tissue the width of attached gingiva at the teeth surface, which invariably leads to recession [8-10]. Accumulation of the plaque on teeth and elements of fixed appliances can be a significant problem [11]. In etiology of recession it is weighty to take into consideration action of occlusal trauma, which enable bacterial

Table 1. **Patients calling for check-ups at the course of fixed appliance treatment resulting from malocclusion and teeth abnormalities**

No	Sex	Age	Fixed appliance treatment duration	Skeletal class ANB / WITS		Incisor position before treatment	Presence of crowding and rotations	Extractions	Favouring factors	Teeth with recession
1	F	23	7 months	I	III	88.91			Gingival biotype	44,43,31,33
2	F	24	6 months	III	III	85.13			Gingival biotype	43,41,31,32,33
3	M	14	12 months	I	III	86.61			Incorrect lip frenulum ↓	41
4	F	16	2 months	III	III	91.89	+		Gingival biotype	45,43,42,41,31,32,33
5	F	17	6 months	I	II	87.65			Gingival biotype	43,42,41,31,32,33
6	F	21	1 year ↓ 18 months ↑	I	I	88.58	+	4+4	Gingival biotype	13,23,33
7	M	24	7 months	I	III	80.51	+		-	43,41,31,32,33
8	F	20	22 months	III	III	78.87	+	4-4, +5	Gingival biotype	43,33
9	F	25	1 month	I	II	93.81	+		Gingival biotype	43,41,31,33
10	M	25	18 months	I	II	91.68	+	4+	Gingival biotype, shallow vestibulum ↓	45,44,43,41,31,33
11	F	13	1 month	I	II	95.63	+	6+, +4, 4-, -6	-	34,35
12	M	12	4 months	II	II	90.80	+		-	41
13	M	39	1 year ↑ 11 months ↓	I	III	105.88	+	4-,+4	Gingival biotype	13,22,23,31,33,35,41,43,45
14	F	22	2 years	II	II	107.57			Shallow vestibulum ↓	44,43,42,41,31,32,33,34
15	M	13	2 years	I	I	102.32	+		Incorrect lip frenulum ↓	41
16	F	20	17 months	I	II	93.92	+	4+4, 4-	-	45
17	F	17	19 months	III	III	85.26	+	4-4	-	43,33
18	M	13	2 years	II	II	97.44	+		-	43,41,33

penetration, favour mechanical irritation during hygienic activities and result in gingival recession [12].

It is widely known that malocclusion deprived the proper treatment increases the risk of caries, inflammatory states of oral mucose, paradontal illnesses and recession. It may be ensued from the clinical observation [13,14] that anterior displacement is critical recession formative agent which may result in substantial gum impairment. Gingival recession may originate before orthodontic treatment, during but also as a result of it. Recession occurring in singular teeth is connected with presence of partial anterior crossbite, especially in patients at young age. Crucial for the rise of recession are also: general condition of soft tissues (too thin gums and improper lip frenulum, tongue, cheek frenulums) [15], bone conditions (thin bone layer on the root or lack of it), teeth related agents (impairment of teeth topography such as crowding or vestibular inclination) [16]. Recession is also frequently observed in supraclusal patients with retrusion of upper incisors or protrusion of lower incisors or few forms of mesioclusion or distoclusion. Patients with complex pathognosic signs, which predispose to recession, should have their teeth relocated in the course of orthodontic treatment inside the alveolar bone [17] and properly selected range of orthodontic forces should be applied in order to execute the relocation [18].

The major aim of this study is to asses the relation between the gingival recession and etiologic factors in patients applying for orthodontic treatment.

Material and methods

The case study group consisted of 52 randomly chosen patients (age range 12-39 years) calling for check-ups at the course of fixed appliance treatment resulting from malocclusion and teeth abnormalities. All the patients were examined in artificial light, with the use of probe, mirror, and paradontometer using criteria consistent with the WHO directives, enclosed in "Oral Health Surveys Basic Methods" [19]. Subsequently acquired data and the values selected from the cefalometric analysis were marked on the table designed for the conducted research according to the scheme: skeletal class according to ANB angle and Wits parameter – showing mutual vertical relations of the jaws, the lower incisor position IMPA, defining the inclination of incisors in relation to the mandibular plane, teeth improperties – crowding, protrusion, dental extractions due to orthodontical indications, periodontium biotype (thin gingiva and the root bone layer), improper upper and lower lip frenulum, shallow oral vestibule.

Among 52 patients 18 were reported, on the basis of interview and clinical records, to have recession. Statistical analysis of obtained data was conducted using variance analysis. Statistically significant were assumed those calculations for which value of significance level $p \leq 0.05$.

Ethics

The Ethics Committee of Medical University of Białystok accepted the study.

Results

The results of the above research are visualized in the *Tab. 1*. Eighteen patients out of 52 had the recession diagnosed which constitutes 34.62%. The total number of teeth with recession was 70, where 5 cases (7.14%) were localized in the maxilla and

Figure 1. **Single recession of tooth 41**

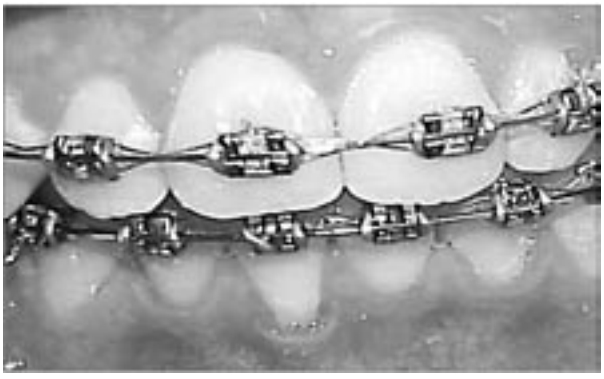


Figure 2. **Multiple recessions of teeth 13, 23, 33, 43, 31, 41**

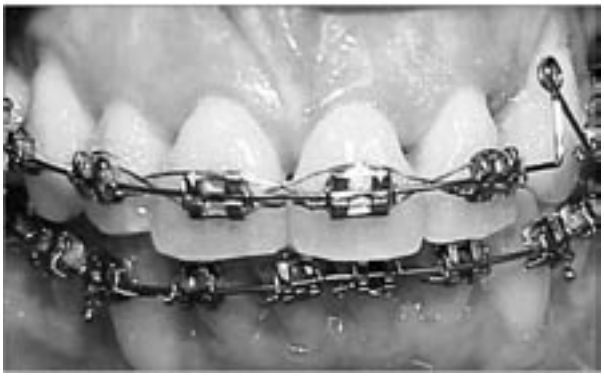
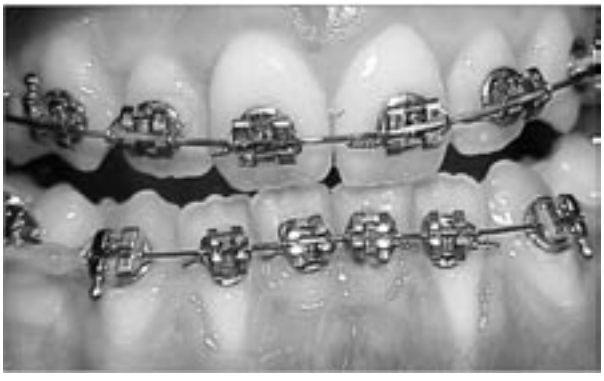


Figure 3. **Gingival recessions of the lateral incisors in the mandible**



65 (92.85%) concerned the mandible. The maxillary recessions comprised mainly canines (4 teeth) and one incisor. The greatest number of gingival recessions in the lower jaw was observed in the anterior segment – 29 teeth (41.42%) and subsequently in the canines – 25 teeth (35.71%). 11 recessions were related to mandibular premolars, which established 15.71% recessions in general. In 5 patients aged 12-14 single recessions occurred *Fig. 1*, whereas in older patients – multiple ones were present *Fig. 2*. Statistically significant differences between the age of patients and recession incidence were estimated ($p \leq 0.003$).

Mutual relation of the jaw basis was described through the analysis of the ANB angle and Wits parameter. In 11 cases (61.12%) the I skeletal class was estimated (ANB=2±3°), 3 cases (16.67%) were related with the II class (ANB>5) and 4 patients (22.22%) were ordered to the III class group (ANB<-1). Normal Wits parameter (0±2 mm) describing the I skeletal class of the jaw basis relationship was found in 2 patients (11.11%). Both Wits II skeletal class (>2 mm) and III skeletal class (<2 mm) was observed in 8 patients (44.44%) respectively. Statistically significant correlation between Wits parameter and gingival recession was estimated.

6 patients (33.33%) represented correct value (90°±3°) of the angle between the lower incisor and the mandibular plane – IMPA. 7 (38.88%) of the orthodontically exposed patients manifested protrusion of the lower incisors (IMPA>93°) and 5 patients (27.77%) – retrusion (IMPA<87°). High percentage (72.22%) of dental irregularities such as crowding and rotations was estimated.

In the course of treatment 17 extractions in 7 patients were performed. Upper and lower first premolars prevailed among the extracted teeth (14 teeth).

The clinical examination of 12 patients (66.66%) revealed presence of recession favoring factors. In 10 patients a single etiologic factor was recognized, 7 patients had two and 1 person had three favoring factors. In the majority of cases one could observe thin gingival and bone plate on the vestibular surface of the root – 9 cases (75.00%) *Fig. 3*. Statistically significant differences between periodontal biotype and gingival recession of teeth 43 ($p \leq 0.048$), 31 ($p \leq 0.016$) and 33 ($p \leq 0.006$) teeth were estimated. The incorrect attachment of the lower lip frenulum was considered to be the main cause of the recession at the tooth 41 in 2 patients (16.66%). These variables, were found to be statistically significant ($p \leq 0.034$). In 2 patients a shallow labial vestibule was recognized to be an etiologic factor for recession appearance in relation to teeth 44 ($p \leq 0.020$), 42 ($p \leq 0.020$) and 34 ($p \leq 0.002$).

Discussion

Epidemiologic research over the gingival recession, prove the illness to be a social problem in the broadest sense of the word [20,21]. Depending on the age of patients, the recession occurs from 6.3 to 100% of population, where the rate of it rises with the age [22], which has also been proved in our research.

Impairment of teeth topography such as crowding or labial positioning of teeth have substantial influence on the formation of the width of attached gums. The recession formation is inclined by the inappropriate positioning of the teeth in the arch. This creates places of poorer resistance of the periodontal tissues resulting from thinning of the gums and narrowing of its width. These are also the potential areas for accumulation of the plaque. Syryńska et al. [23] in her 6 year research over children proved that rotations, proclination and crowding of teeth significantly narrowed the attached gums width in particular teeth. Clinical research of our group (age range 12-14 years) has also proved recession in particular teeth resulting from dental inaccuracies.

The most adverse influence had the teeth with vestibular inclination in the jaw. Such situation is connected with lowering of the bone and gum thickness which propped up by the impair-

ment ensuing from intense brushing inevitably results in recession of gingiva. In the analyzed data protrusal positioning of the lower incisors were documented in 38.88% of the trial group. Louis A.Buckley [24] and few other researchers revealed the reliance between the crowding of the lower incisors, their vestibular inclination and the impairment of the gums. They have also proved crowded teeth to be far more difficult to clean thus prevent the plaque. All these factors inevitably lead to impairment of periodontium. 13 out of 18 patients with diagnosed recession had their dental inaccuracies reported. They called regularly for check-ups during their fixed appliances treatments. The whole group has undergone a detailed oral cavity hygiene instruction before and during the check-ups, so the plaques together with dental inaccuracies are doubtful etiological factors in the diagnosed cases of recession.

Excessive recessions of the marginal periodontium are also observed in deep bite cases with retrusion of upper incisors and protrusion of lower incisors, or few forms of mesioclusion or distoclusion [25].

Geiger [26] in his work concerning orthodontic treatment and associated periodontological problems presents slightly different perception of gingival recession. He invariably links the disease with too thin gingiva and bone layer covering the root, impairment of teeth topography, shallow labial vestibule and incorrect labial frenulums. Anatomic factors together with pathological attachments of low labial frenulums, shallow vestibule or high mentalis muscle attachment, where increased tissue tension occurs, favour recession manifestation. In the study group described, the most common multiple recession etiologic factor were thin gingiva and bone plate covering the root as well as shallow lower vestibule, whereas single recession were related to the incorrect attachment of the lower lip frenulum.

The described study particularly attached importance to the Wits parameter, characterizing the linear relationship of the jaw basis. In our work the III skeletal class occurrence corresponded with the presence of one etiologic factor – mainly biotype. Thin bone and gingiva covering the root from the vestibular surface are quite often a characteristic feature of periodontium in patients with the III skeletal class, particularly in cases with retrusive lower incisors.

Decrease of the IMPA values below 87° thus lingual proclination of the lower incisors may occur independently from the skeletal component or exist as a form of dental compensation of the skeletal irregularity.

Conclusions

Anatomic factors together with dental and occlusal improperties may originate singular or multiple recessions. Orthodontic patients calling for treatment due to malocclusion should be under professional supervision when symptoms suggest the recession occurrence especially when clinical examination may diagnose already existent gingival recession.

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Complications in the course of surgical-orthodontic treatment of impacted maxillary canines

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Abstract

Purpose: The purpose of the study was to assess the effect of gender and age of patients with impacted permanent maxillary canines on complications in the course of tooth transposition.

Material and methods: The study material included files of 82 patients with a diagnosis of unilateral or bilateral impaction of 102 permanent maxillary canines. The study group consisted of 65 female and 17 male subjects, aged 8.5-39 years (mean 14.5 years) divided into four age groups: group I – patients under 12, group II – 12.0-13.9 years, group III – 14.0-15.9 years and group IV – patients at the age of 16 and older.

Results: In the study population, the impacted teeth showed the following locations: palatal (67.64%), vestibular (19.60%) and alveolar (12.74%). Spontaneous resorption caused by abnormal tooth position was observed in 5 (4.9%) permanent maxillary lateral incisors. In 4 cases, the resorption was bilateral and all the five cases were recorded in group III. In group IV, one patient had alveolar process atrophy and severe resorption, while another one showed ankylosis of a permanent canine. Extraction of palatally impacted canines was done in 3.92% of cases. Complications were noted in girls and referred to 5.58% of the study cases.

Conclusions: Orthodontic movement of the impacted teeth to the dental arch may result in complications. However, because of the major significance of the upper canine which is responsible for the behaviour of the frontal triad, surgical-orthodontic treatment should be undertaken to improve occlusion and the aesthetic look of patients. Thus, any case of the ectopic canine requires observation and proper choice of radiological diagnostics.

Key words: impacted permanent upper canines, root resorption, ankylosis, therapeutic methods.

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Introduction

According to literature data, the incidence of maxillary canine impaction ranges between 1% and 3% of the population [1-4]. Palatal impaction is the most frequent (85%) [5]. As stated by Shafer [6], leaving the retained canines untreated may result in serious sequels: displacement of the adjacent teeth and shortening of the dental arch, internal resorption, formation of follicular cysts, external resorption of the canine and the adjacent teeth, recurrent infections especially when the tooth is partially erupted, recurrent pain, or combinations of the above. Transposition of the impacted teeth may be accompanied by a number of negative events, including periodontal ailments that manifest themselves in the loss of bone retention, inflammation of the marginal gingiva, deepening of periodontal pockets, resorption of the canine and/or the adjacent teeth, root shortening, internal resorption, ankylosis and even tooth loss [7-11].

The aim of the study was to evaluate the effect of gender and age of patients with impacted permanent maxillary canines on complications in the course of orthodontic movement to the occlusal plane.

Material and methods

The study material included documentation files of 82 patients with a diagnosis of unilateral or bilateral impaction of 102 permanent maxillary canines. The study group consisted of 65 female and 17 male subjects, aged 8.5-39 years (mean 14.5 years) divided into four age groups: group I – patients under 12,

Table 1. **Study population with regard to gender and age**

Sex	Age (years)				Total
	I	II	III	IV	
	< 12	12 ≥ 13.9	14 ≥ 15.9	≥ 16	
	n	n	n	n	n
Female	9	14	17	25	65
Male	3	4	5	5	17
Total	12	18	22	30	82

Table 2. **Distribution of locations of the impacted canines with regard to age**

Age groups	Canines	Canines locations				
	n (%)	Palatal	Vestibular	In the process	Right-side	Left-side
		n (%)	n (%)	n (%)	n (%)	n (%)
I	19 (18.26%)	4 (3.92%)	6 (5.88%)	9 (8.82%)	10 (9.80%)	9 (8.82%)
II	20 (19.60%)	15 (14.70%)	5 (4.90%)	-	11 (10.78%)	9 (8.82%)
III	28 (27.45%)	20 (19.60%)	4 (3.92%)	4 (3.92%)	10 (9.80%)	18 (17.64%)
IV	35 (34.31%)	30 (29.41%)	5 (4.90%)	-	14 (13.72%)	21 (20.58%)
Total	102 (100.00%)	69 (67.64%)	20 (19.60%)	13 (12.74%)	45 (44.11%)	57 (55.88%)

n – number of the canines; data are number %

Table 3. **Complications caused by transposition of the impacted canines with regard to age**

Groups	Age	Patients	Complications		
			Lateral incisors roots resorption	Root canine resorption	Ankylosis of the permanent canine
		n (%)	n (%)	n (%)	n (%)
I	<12	12 (14.63%)	-	-	-
II	12 ≥ 13.9	18 (21.95%)	-	-	-
III	14 ≥ 15.9	22 (26.82%)	5 (4.9%)	-	-
IV	≥ 16	30 (36.58%)	-	1 (0.98%)	1 (0.98%)
Total		82 (100.00%)	5 (4.9%)	1 (0.98%)	1 (0.98%)

n – number of the patients; data are number %

group II – 12.0-13.9 years, group III – 14.0-15.9 years and group IV – patients at the age of 16 and older (*Tab. 1*).

Statistical analysis

Results were subjected to statistical analysis, using t-Student test, non-parametric Wilcoxon test and Pearson correlation coefficient for paired variables.

Ethics

The Ethics Committee of Medical University of Białystok accepted the study.

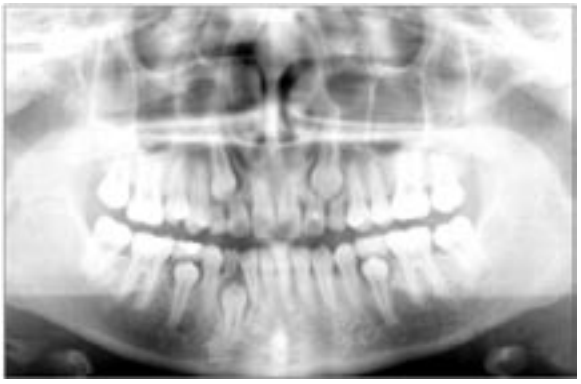
Results and discussion

In the study population, the impacted teeth had the following locations: palatal (67.64%), vestibular (19.60%) and in the process (12.74%). The position of the retained teeth was age-dependent – in the youngest patients most teeth (9) were impacted in the process, while in patients over 12 most teeth showed palatal position. Right-side location was noted in 44.11% and left in 55.88% of the impacted canines. Right-side retention was predominant in patients from groups I and II, while left-side retention was found to prevail in groups III and IV (*Tab. 2*). Complications caused by the impaction of permanent maxillary canines have been described in *Tab. 3*. Spontaneous resorption due to abnormal tooth position was observed in 4.9% of the permanent maxillary latera incisors. In 4 cases of group III, the resorption was bilateral (*Fig. 1A-C*). In group IV, one patient had atrophy of the alveolar process and severe resorption (*Fig. 2*) and another one showed ankylosis of the permanent canine. Extraction of palatally impacted canines was done in 3.92% of cases. Complications were noted in girls and referred to 5.58% of the study cases.

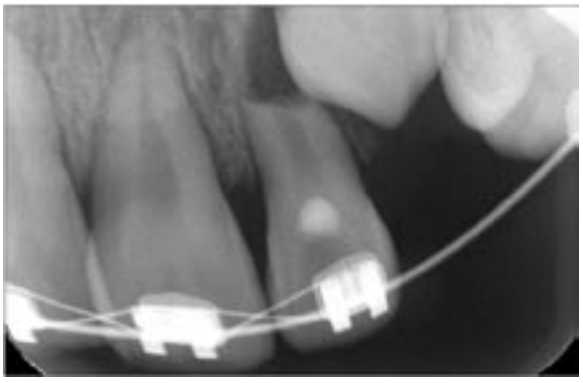
Retention of permanent maxillary canines incurs the risk of complications, among which resorption of permanent incisors is quite frequent. Early diagnosis of resorption is difficult as in most cases it occurs posteriorly or anteriorly in the mid-root of the lateral incisor. Studies conducted by Ericson and Kurol [3,12-14] have demonstrated the necessity to take X-ray pictures not later than at the age of 10 to supplement the clinical examination whenever resorption is suspected. If in the oral cavity examination, lateral incisors exhibit atypical position, i.e. the crowns are distally protruding and deviated, and canine ridge is

***Figure 1A-C*. Panoramic radiograms and radiogram of a 14.5-year-old girl with distinct advanced tooth resorption 12, 22**

a)



b)



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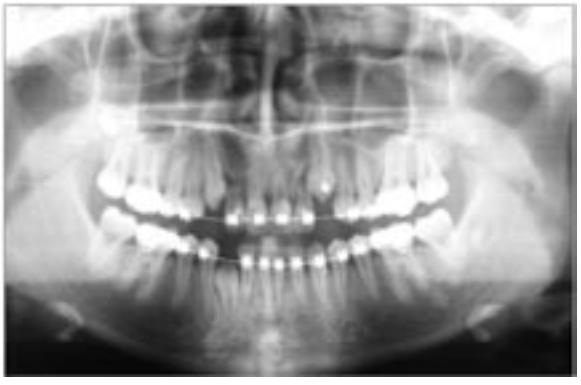
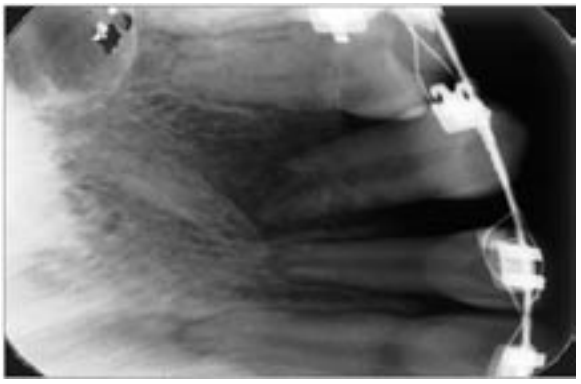


***Figure 2*. Contiguous radiogram of the left maxillary canine with the loss of bone retension and root resorption 23**



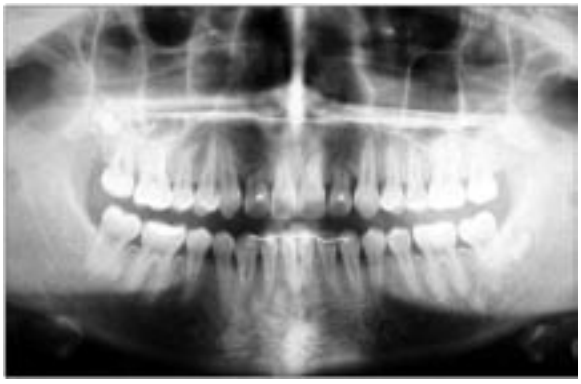
palpable, resorption can be recognised. The incidence of resorption amounts to 12.5%, and thus total percentage of cases in the age range of 10-12 years is 0.7% [3].

Resorption of lateral incisor roots is progressing rapidly and is unpredictable. The canines remaining in the process have a close contact with lateral incisors and cause loss of cortical plate, which is visible in radiograms. Heins and Wieder [15] have found that the spongy bone disappears when the interval between the roots is smaller than 0.5 mm and then teeth get in a direct contact. Shafer et al. [6] observed permanent incisor root resorption caused by ectopic eruption of maxillary canines in 0.71% of children aged 10-13 years. This complication has irreversible sequels, including tooth loss. Ericson and Kurol [12], who analysed cases of lateral incisor root resorption, have demonstrated that the ectopic eruption itself does not increase the risk of resorption. Other predisposing factors that may induce this process include: mesial position of canine crown, advanced development of canine, increased mesial ectopic eruption pathway above 25 degrees as compared to the median osseous line and age of 11-12 years. Resorption occurs mainly in girls [6,12,16]. Thanks to the availability of computerized tomography a larger number of resorption cases can be recognized than through clinical examinations [17,18]. In their latest study on a group of 80 patients with 113 impacted canines and marked resorption of 39 incisors, Bjerklin and Ericson [7] changed the treatment plans in 35 patients, extracting damaged incisors in cases which needed extraction due to space deficiency. The decision to change the treatment was made after a year, following additional computerized tomography examination.

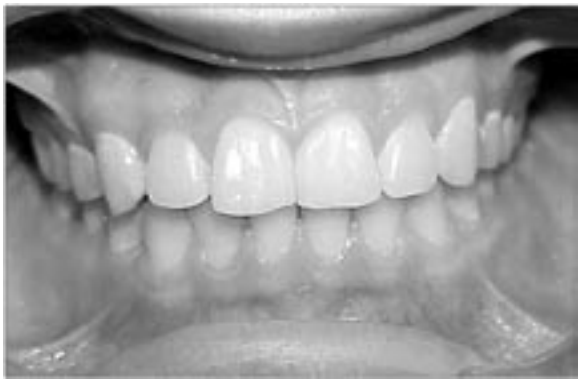
We found resorption of 5 incisor roots in 3 female patients in the age range of 14-15.9 years. In four cases, resorption was revealed by the first radiogram, in the fifth case this process, which was caused by the impacted canine, was seen only when the tooth was shifted to the arch and did not blur the resorption image any longer. In all the cases of resorption, the lateral incisors had abnormal position, showing labial protrusion and distal inclination. In two female patients, linear values indicated deep location of the impacted canines. In the third patient, with advanced absorption, the canines were close to the occlusal plane and there was bilateral space deficiency in the arch and total left-side crossbite occlusion. Having in mind that the direction of canine eruption pathway changes with personal development it is difficult to indicate the moment at which the location of the erupting tooth is likely to initiate the process of lateral canine resorption. The increased risk of resorption in girls [12] has been confirmed by our study and should be always considered. The diagnosis of the impacted canine accompanied by resorption of lateral incisor roots requires immediate separation of both teeth in order to stop resorption progression. Examinations of 5 resorbed lateral incisors confirmed their vitality and resorption arrest. These observations are consistent with the data reported by Becker and Chaushu [19], who performed a comparative study in order to evaluate resorption progression in the incisors in which severe resorption was related to maxillary canine retention. *Fig. 3A-B* shows a panoramic radiogram

Figure 3A-B. **Intraoral radiogram and panoramic radiogram of a female patient after completion of surgical-orthodontic treatment**

a)



b)



and intraoral picture of a patient with lateral incisor resorption caused by a 2-year retention of the maxillary canine. The teeth have not undergone endodontic treatment, are vital and in proper colour.

Surgical-orthodontic treatment of the impacted teeth may also have an undesired effect on the alveolar bone and on the root of the transpositioned teeth. Serious resorption of a permanent canine in a 19-year-old patient coexisting with radiologically diagnosed atrophy of the alveolar process was due to the reaction against orthodontic force involved in a 180° tooth rotation in the alveolar process. In the study material, 4 out of the 102 impacted teeth had to be chiseled out. In one case, this procedure was necessary due to abnormal tooth position and advanced age of the patient (39 years), in another patient tooth transposition failed and the revision of the site of retention revealed ankylosis, in the other two cases extraction was indicated to improve occlusion, i.e. the contact between tooth four and two ensured both functional and esthetic occlusion. In such cases, canine extraction, according to Masztalerz [20], is the best option. However, as revealed by Suri et al. [21], extraction is not recommended in the case of vestibular retension of the canines, since surgical intervention can damage soft tissues and bones, causing scar formation on the alveolar process and thus worsening aesthetic appearance of the frontal segment of the dental arch.

Conclusions

Alignment of the impacted teeth may result in complications. However, considering major significance of the maxillary canine responsible for the behaviour of the frontal triad, it seems necessary to undertake surgical-orthodontic treatment in order to ensure proper occlusion and improve aesthetic appearance. Any case of ectopic eruption of the canine requires observation and proper choice of radiological diagnostic techniques.

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Tryptophan and its metabolites in patients with oral squamous cell carcinoma: preliminary study

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Abstract

Purpose: It has been showed that tryptophan (TRP) degradation has been linked to modulation of cancer cell proliferation. The aim of our study was to estimate the concentration of TRP and its derivatives, such as anthranilic (AA) and kynurenic acid (KYNA) in plasma, saliva, squamous cell carcinoma (SCC) tissues and healthy oral mucosa in patients with oral SCC.

Material and methods: The study was performed on plasma, non-stimulated, mixed saliva and squamous cell carcinoma tissues and healthy oral mucosa in patients with oral SCC. The concentration of TRP and its metabolites were determined by high-performance liquid chromatography (HPLC).

Results: In plasma the concentration of TRP was 33.73±2.52 μM, of KYNA was 26.97±5.35 nM and of AA was 32.40±2.30 nM. In saliva the concentration of TRP was 3.81±0.62 μM, of KYNA was 8.06±1.86 nM and of AA was 20.41±10.77 nM. In cancer tissues the levels of TRP (30.21±5.88 μM), KYNA (15.85±1.82 nM) and AA (265.32±151.45 nM) were higher in respect to the concentration of TRP (13.28±0.62 μM), KYNA (12.75±2.28 nM) and AA (31.68±8.89 nM) in normal tissues. The increase in the content of TRP, KYNA and AA in cancer tissues reached 127.48±5.95%, 24.31±4.35% and 737.50±206.96%, respectively.

Conclusions: Our study has demonstrated the change of TPR metabolism, which is reflected by the increase TRP, AA and KYNA concentrations in patients with oral squamous cell carcinoma. We can suppose that these substances may be one of many factors responsible for cancer development.

Key words: oral cancer, tryptophan metabolites.

Introduction

Cancers of the oral cavity represent approximately 2.5% of all malignant neoplasms in Poland. Squamous cell carcinoma (SCC), which arises from the oral mucosal lining, accounts for over 90 percent of these tumors [1,2]. The most common site for oral carcinoma is the tongue, which accounts for around 40 percent of all cases of caries of the oral cavity proper. These tumors most frequently occur on the posterior lateral border and ventral surface of the tongue. The floor of the mouth is the second most common oral location. Less-common sites include the gingiva, buccal mucosa, labial mucosa and hard palate [3].

Despite of advances in surgery, radiotherapy, and chemotherapy, the five-year survival rate among patients with oral cancer has not improved significantly over the past several decades and it remains at about 50 to 55 percent [4]. So there is a need for more data which not only will help to improve new therapeutic oncologic modifications but also will be useful for finding potential substances to easier diagnose, treat and monitor of oral cancer.

L-tryptophan (TRP), essential amino acid, is metabolized in 95% via kynurenine pathway [5]. The first of TRP metabolite is N-formylkynurenine which is further catabolized to kynurenine (KYN) by constitutive intracellular formylase. KYN is transformed to a number of metabolites such as anthranilic (AA) and kynurenic acid (KYNA), which are biological active substances. AA plays an important role in the regulation of immunological processes [6,7] as well shows antibacterial activity [8]. However, KYNA has been identified as an essential neurotransmitters' agonist [9,10].

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Table 1. **Baseline characteristics of patients**

	Patient 1	Patient 2	Patient 3	Patient 4
Age (yrs)	49	52	81	45
Gender	F	M	M	M
Diagnosis	Carcinoma planoepitheliale keratodesa G_2	Carcinoma planoepitheliale keratodesa G_2	Carcinoma planoepitheliale keratodesa G_2	Carcinoma planoepitheliale keratodesa G_2
Localization	Carcinoma fundi cavi oris sinistri	Carcinoma buccae dextri	Carcinoma buccae dextri	Carcinoma linguae dextri
Staging	$T_3N_{2A}M_0$	$T_4N_{2B}M_0$	$T_4N_1M_0$	$T_3N_1M_0$
WBC [$10^3/\mu l$]	6.0	6.42	6.6	6.6
RBC [$10^6/\mu l$]	4.63	3.90	4.44	4.54
HGB [g/dl]	14.6	12.8	12.6	14.6
HCT [%]	43.3	36.5	37.8	42.6
PLT [$10^3/\mu l$]	196	242	339	324
ISE Na^+ [mmol/l]	130	140	142	140
ISE K^+ [mmol/l]	3.76	4.50	3.83	4.50

The literature has showed that AA can induce liver cancer in mouse offspring, which mother had administered AA transplacentally [11]. Moreover, the increased concentration of AA was found in urea of patients with bladder cancer. This observation and later studies have proved that AA is potential carcinogen [12].

Because of data have showed that TRP degradation has been linked to a modulation of proliferation of cancer cells we decided to study it level in plasma, saliva and tissues (normal and cancer) patients with oral carcinoma, which are of the most frequent. Nevertheless, there are many studies in this subject, the pathogenesis of this disorder is still unknown.

In our previous study we have found TRP metabolites in human saliva. At the same time we have not observed any changes in indoleamino 2.3-dioxygenase activity, enzyme responsible for TRP metabolism, in saliva of these patients [13].

Thus, the aim of our study was to estimate certain TRP derivatives in plasma, saliva and salivary glands (normal and cancer) in patients with oral carcinoma.

Material and methods

Specimen collection and patient details

Baseline characteristics of the patients who were included in the study shows *Tab. 1*. These patients met the following criteria: absence of other diseases in which production of saliva is impaired (including: diabetes, Sjögren syndrome) and there was no administration of any pharmaceuticals which could affect saliva production. None of the patients had received blood transfusion for at least 3 months or any drugs which could affect the function of the immune system. The TNM classification according to UICC convention was used to evaluate clinical tumor stage. All patients were informed about the aim of the study. Written consent was obtained from each subject and Local Ethical Committee approved the experimental protocol.

A cancer tissue specimens were obtained from patients who were classified for surgical treatment and underwent surgery at the Department of Maxillofacial Surgery Medical University of Białystok, Poland. Normal epithelium (control tissue) was received from the margin of these resections of these carcinomas.

Samples were homogenized in ice-cold with 2 M TCA and centrifuged at 12000 g for 15 minutes at 4°C. The supernatant fluid was passed through a WATERS 0.45 μM filters. The concentrations of TRP, KYNA and AA were determined by high-performance liquid chromatography (HPLC) [14].

Blood sampling

Venous blood was drawn in the morning between 7 and 8 am and put into a tube containing 3.8% sodium citrate (citrate/ /blood =1:9). Hematological (red and white cells count, hematocrit, hemoglobin, plates) and biochemical (the concentrations of sodium and potassium) parameters were assayed by standard laboratory methods.

In order to estimate tryptophan, kynurenic and anthranilic acid concentration, the plasma was deproteinized with 2 M $HClO_4$ and centrifuged at 12000 g for 15 minutes at 4°C. The supernatant fluid was passed through a WATERS 0.45 μM filters. Samples were stored at -80°C until assayed. TRP and its metabolites were determined by high-performance liquid chromatography (HPLC) [14].

Saliva sampling

Samples of non-stimulated mixed saliva were taken from patients each morning between 7 and 8 am, 10 min after mouth washing MilliQ water using the spitting method. The saliva samples were immediately treated 2 M $HClO_4$ and after 15 min of incubation with acid at 4°C, samples were centrifuged 30 min 12000 g. The supernatant was collected in -80°C for measurement of concentrations of TRP and its products degradation by high-performance liquid chromatography (HPLC) [14].

Statistical analysis

The values are expressed as the mean ±SEM or as a real values.

***Table 2*. The real values of TRP and its metabolites as well the ratio of KYNA/TRP and AA/TRP in patients with oral squamous cell carcinoma**

		Patient 1	Patient 2	Patient 3	Patient 4
TRP	plasma [nM]	27.91	31.10	38.10	37.79
	saliva [nM]	5.03	4.68	2.43	3.09
	control tissue [pmol/g tissues]	14.98	13.30	11.66	13.48
	carcinoma tissue [pmol/g tissues]	43.83	18.67	36.03	22.29
KYNA	plasma [nM]	36.38	22.32	35.14	14.05
	saliva [nM]	9.92	10.32	6.20	12.67
	control tissue [pmol/g tissues]	17.91	6.89	12.40	13.78
	carcinoma tissue [pmol/g tissues]	15.16	17.91	11.02	19.29
KYNA/TRP ratio	control tissue	1.20	0.52	1.06	1.02
	carcinoma tissue	0.35	0.96	0.31	0.87
AA	plasma [nM]	12.67	9.50	11.09	9.50
	saliva [nM]	12.45	40.92	15.84	4.48
	control tissue [pmol/g tissues]	52.80	10.56	36.96	26.40
	carcinoma tissue [pmol/g tissues]	253.44	36.96	696.96	73.92
AA/TRP ratio	control tissue	3.52	0.79	3.17	1.96
	carcinoma	5.78	1.98	19.34	3.32

***Figure 1*. The increase of TRP and its metabolites in cancer tissues**

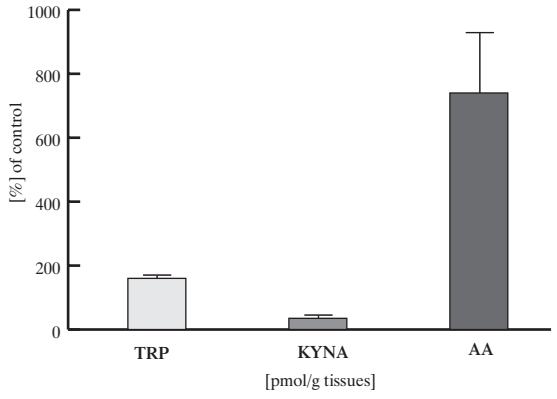
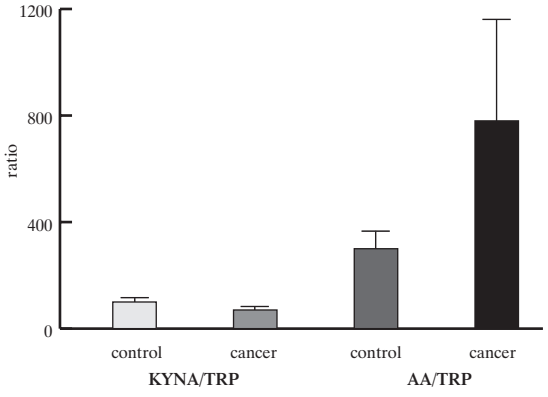


***Figure 2*. The ratio of KYNA/TRP and AA/TRP in cancers tissues**



Results

The estimation of TRP and its metabolites via degradation of kynurenine pathway is presented in *Tab. 2*. In plasma the concentration of TRP was 33.73±2.52 μM, of KYNA was 26.97±5.35 nM and of AA was 32.40±2.30 nM. In saliva the concentration of TRP was 3.81±0.62 μM, of KYNA was 8.06±1.86 nM and of AA was 20.41±10.77 nM. In cancer tissues the levels of TRP (30.21±5.88 μM), KYNA (15.85±1.82 nM) and AA (265.32±151.45 nM) were higher in respect to the concentration of TRP (13.28±0.62 μM), KYNA (12.75±2.28 nM) and AA (31.68±8.89 nM) in normal oral mucosa. The increase in the content of TRP, KYNA and AA in cancer tissues reached 127.48±5.95%, 24.31±4.35% and 737.50±206.96%, respectively (*Fig. 1*).

The ratio of KYNA/TRP was 95.00±14.84 in control tissue (normal epithelium) and 62.25±17.01 in cancer tissue. The ratio of AA/TRP was 236.00±63.09 in control tissue and 760.50±399.00 in cancer tissue (*Fig. 2*).

Discussion

For the first time we have observed the increase of TPR and its metabolites in the human oral cancer tissues. Among the all studied substances the enhanced of AA was the highest. Its concentration was almost 8.5 times higher in cancers' tissues in comparison to level observed in normal tissues. This high concentration, observed only in tumor, suggests that AA can be produced by cancer cells. In the literature is not any information about a role of this substance in oral carcinogenesis.

However, the data showed that AA has been implicated in carcinogenesis of the liver [15]. This observation is in line with the finding of Fujii and Watanabe who have demonstrated that transplacentally administration of AA for 1 year induced liver tumor in male mouse offspring [11]. In 1960s years several groups of workers suggested that AA has got carcinogenetic properties and it is responsible for development of human bladder cancer [12]. The authors confirmed, if bladder cancer is caused by agents presented in the urine, e.g. AA, it should be

also involved in the protective mechanism against carcinogenesis [12]. Thus, on the one hand the high level of AA can be a way of protection of cancer cells against their own toxins, on the other hand it can be implicated in oral carcinogenesis. The mechanism of activity of AA against cancer development is probably used in anticancer therapy according to the last data which has showed that effect in a new compounds designed as the anthranilic acid scaffold. The antiproliferative activity of AA was observed in vitro and in vivo in cancer experimental models [16,17].

We have also observed the elevation of TRP concentration almost 2.3 times in the oral carcinoma tissues in comparison with control healthy mucosa. Since TRP is known as an essential amino acid necessary for a variety of metabolic processes, e.g. protein biosynthesis and its availability and also it is necessary for rapidly-dividing tumors [18]. Thus, observed accumulation of TRP concentration in our study may be explained as self-protection of tumor cells.

KYNA concentration has showed the lowest increase in cancer tissues. Physiologically it is an antagonist of ionotropic glutamate receptors. Recent it was found that glutamate antagonists inhibit a proliferation of different human tumor cells [19]. We cannot exclude that small increase of KYNA concentration may be result of inhibition of its synthesis by cancer cells and thereby it can be a form of self-protection. So, it can suggest that KYNA is involved in regulation of carcinogenesis as well other disorders. Our recent study has showed the increase of saliva concentrations of KYN and KYNA in patients with both diabetes and hypertension in comparison to healthy volunteers and patients with hypertension, or diabetes alone [13].

In conclusion, our study has demonstrated the change of TRP metabolism which has been reflected by the increase of TRP, AA and KYNA concentrations in patients with oral squamous carcinoma. Moreover, we have showed the shift of amino acid transformation pathway on AA side. Our results have indicated accumulation of these substances in squamous cell cancer. Because their concentrations are lower in plasma and saliva we cannot exclude that cancer cells synthesize both of them locally. Thus, we can suppose that these substances may be one of many factors responsible for cancer development. Moreover, further studies are needed to prove it.

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The clinical assessment of mobile teeth stabilization with Fibre-Kor

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Abstract

Purpose: A glass fiber tapes are used in periodontal diseases to stabilize mobile teeth. The purpose of this project was to make a clinical appraisal of teeth stabilization which were using Fibre-Kor splinting.

Material and method: 56 patients 35-67 year old were examined. There were made 162 teeth blocks using Fibre-Kor as reinforcement and Flow-It material as matrix. After 10 months clinical parameters such as: PI, SBI, GI and periodontal pocket were checked.

Results: Periodontal pockets depth decreased average by 0.58 mm after teeth stabilization. Bleeding index and inflammation of gums fall average by 2.55 and 1.95. The average oral cavity hygiene improved and achieved 1.46.

Conclusion: The Fibre-Kor splint is an esthetic and functional solution of mobile teeth stabilization, and is a part of the specialist periodontal treatment.

Key words: periodontal diseases, pathological teeth mobility, splinting, Fibre-Kor.

Introduction

Teeth mobility is one of the periodontal disease symptoms. This mobility comes as a result of bone destruction which causes disfunction, traumatic occlusion and in the end loss of the teeth. Stabilization is one of the conditions which allow to save our own teeth. There were used many methods of stabilization in the history of dentistry. This methods were not efficient because they didn't last for a long term. Through that they didn't help to heal the structures of the periodontal tissues which were still mobile [1-3,5-7,10].

Glass fiber came to dentistry in the 90's of the XX century. It revolutionized the treatment of the mobile teeth. There are many different solutions in dentistry. It is difficult to choose appropriate one because of a wide range of the materials and different clinical situations [2-4,6-8,10,11].

The Aim

The purpose of this clinical study was to make an appraisal of the clinical parameters of stabilized with Fibre-Kor teeth.

Material and methods

The research concerned 56 patients (32 female and 24 male). They were 35 to 67 year old. 47 of them had chronical periodontal diseases, and 9 of them agressive. There were made 162 teeth blocks which connected from 2 to 6 teeth. There were qualified to splinting teeth with IIo and IIIo mobility Entin scale. The stabilization was made by connecting mobile teeth with stabile onces on both sides. One of the rules of stabilization says that we should connect maximum amount of teeth. The splint should be settled in static balance points. It should consider changes of the biostatic caused by lengthen of clinical crown. The splint cannot lay on the gums to allow hygienic procedures being made. The ability of keeping in good condition dental hygiene and esthetic expectation were estimated before treatment. All of the patients were motivated and trained in dental hygiene care. The tartar was removed, root planning and initial bite correction was made. The assessment such as: Silness and Löe PI index, Mühlemann and Sone SBI index, Löe and Silness GI index and depth of the periodontal pocket was made. Fibre-Kor splint which consists

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Table 1. **Average clinical parameters before and after 10 months of stabilization**

Indexes	Initial assessment	Assessment after 10 months
PI	2.62	1.16
SBI	4.42	1.87
GI	2.93	0.98
Pocket depth	5.11	4.53

of a group of parallel preimpregnated with resin glass fiber was used for splinting. Stabilized group of teeth was prepared on the occlusion surface of molars and premolars and lingual surface of incisors and canines. The next step was etching of contact and prepared surfaces, then the bonding was made with Bond 1 from Pentron. Appropriate length of Fibre-Kor was settled with Flow-It from Pentron in prepared groove and light cured. Then then splint was willed again with flowable composite. The last step was the bite correction. After 10 months the assessment of clinical parameters was made. In mean time the patients came for a monitoring visits.

Results

Results of the research was shown in the *Tab 1*. Average value of the clinical parameters before and after 10 months from stabilization. All of the parameters decreased:

- PI – from 2.62 to 1.16;
- SBI – average decrease of SBI index was 2.55
- GI – average decrease of GI index was 1.85.
- Depth of the periodontal pockets decreased average about 0.58.

Discussion

Clinical revives says that more esthetic, acceptable and functional are inside teeth splints. The same opinion had patients who were using both kind of stabilization. A significant thicken of teeth surface after using Fiber-Splint, especially in the front of the upper jaw. This situation may raise the occlusion height which causes destruction of the splinting [6,7,10,8]. Fibre-Kor splinting is an excellent solution because it's preimpregnated fibres connects with great strength with flowable material (Flow-It). This materials in our experimental examinations had the best mechanical properties [4,9,11]. The fault of this splint is preparation of hard toot tissue. All of the monitored parameters had improved in all cases. Starting PI was twice higher so worse. Despite of frequent dental hygiene training and monitoring visits we didn't reached as low values as the other researchers who reached lower then 1 PI index [6,10]. GI and SBI indexes were also higher than at the other researchers patient's. However, the monitoring examinations shown good treatment results. Average decrease of SBI index was 2.55; GI decreased about 1.85. Those effects are excellent in comparison with the other researchers patient's. Average values which they noticed were: 0.4 to 1.09 (SBI); 0.14-0.15 (GI). We have noticed average reduction of the depth of periodontal pockets about 0.58 which is correlated with the other authors raports, who noticed values about 0.17 and 0.65 [6,10]. The clinical improvement of the examined patients cases was possible because of intensive hygienic treatments often monitoring visits and specialist periodontal treatment. We focused our attention on a bite correction before and after splinting. This is a part of interdisciplinary periodontal diseases treatment which helps us make it successful. We should remember, that the teeth stabilization should be the beginning to the next steps of the specialist periodontal treatment, because it eliminates just the symptoms not the cause. When we leave infected periodontal pocket, we condemn unaware patient for further development of the illness [1,5-9].

Conclusions

1. The shown method is modern, effective, durable and esthetic which gives back the function of mobile teeth. It is exemplary in selected clinical situations.
2. The repair and hygiene care is very easy.
3. Stabilization of mobile teeth with specialist periodontal treatment significantly improves periodontal clinical parameters.

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The clinical and radiological assessment of periodontal bone loss treatment using Emdogain

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Abstract

Admission: **Emdogain is the only one biomaterial using biomicra effect which is practiced in periodontal surgery.**

Purpose: **The purpose of the study was a clinical and radiological assessment of bone loss treatment using Emdogain.**

Material and methods: **There were 19 persons examined (11 women and 8 men) which have bone loss treated. Initial and monitoring examination after 10 months embraced clinical parameters such as PPD, CAL and radiological – based on intraoral x-ray pictures. Emdogain treatment was made according to surgical procedures.**

Results: **The research has shown reduction of the depth of periodontal pockets average about 3.4 mm and attachment connective tissue growth about 2.2 mm. Bone loss filling was on 67.1% level.**

Discussion: **Bone loss filling and growth of connective tissue attachment are in our research lower than in most of the others publications. Our observation concerned 10 months period so we should expect better effects after longer time.**

Motions: **Emdogain is safe and effective regeneration material.**

Key words: Emdogain, bone loss, bone regeneration.

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Admission

Nowadays periodontology offers a wide range of biomaterials used for regenerations of destructed by illness periodontal tissues. Only one of them uses biomicra effect [2]. This is an Emdogain which duplicates a physiological process in embryogenesis of cement, periodontium, alveolar process. This is a substance which contains amelogenin albumen. This preparation creates excellent conditions to selective absorption and migration of the cells periodontium and building a new connective tissue attachment on cleaned up surface of the root. Through that it rebuilds lost periodontal tissues [1,5,7,9,14,15]. Only Emdogain causes formation of the new cellules with a multiple amount of egzogenic fibers. It also protects before formation of endogenic fibers. There are suggestions that Emdogain can cause very advantageous effect after periodontological microsurgical treatment.

The Aim

The aim of the research was the clinical and radiological assessment of bone loss treatment using Emdogain.

Material and methods

There were 19 persons examined (11 women and 8 men) in 29-34 year old age. They were general sound and non smokers. There were found 42 interproximal cavities (28 3-wall and 14 2-wall) which were assessed during operations.

Clinical and radiological review was carried before treatment and after 10 months. In clinical examination teeth mobility, depth of periodontal pockets (PPD in mm), level of clinical attachment (CAL in mm) was considerated. Destruction level of dental process was estimated according to intraoral x-ray pictures (linear measurement of the depth and width of bone loss). In the initial phase of the treatment oral cavity hygiene training

Table 1. **The average clinical parameters values PPD, CAL before and after 10 months from microsurgical periodontal treatment**

	PPD (mm)	CAL (mm)
Before treatment	8.5	10.4
10 months after treatment	5.1	8.2
Improvement	3.4	2.2

Table 2. **The average values of radiological index before and after months from microsurgical periodontal treatment**

	depth of bone loss (mm)	width of bone loss (mm)
Before treatment	7.8	3.3
10 months after treatment	4.7	1.8
Bone growth	3.1	1.5
Defect filling percentage	67.1%	

was carried out. Calculus was removed and root planning was made. At the end bite correction was carried out. Mobile teeth were splinted by Fibre-Kor splinting. Surgical treatment was carried out in local anesthesia. After the cuts in periodontal pockets were made muco-gingival flap was exposed (at the vestibular, lingual or palatial side). The vertical cuts was made only there where it was necessary to allow better access or better wound closing. The granular structure was removed, scaling and smooth of the root was made. The operation field was rised out with 0.9% NaCl. After mechanical preparation of the root surface Emdogain was carried in starting from apical part. The muco-periosteal flap was reposition to the crown side and sawn with non-reabsorbable suture according to producer directions. After 2 or 3 weeks stickes were removed. The patients were recommended to: rinse oral cavity with 0.2% chlorhexidine solution, use antibiotics for 7 days and monitoring visits with professional teeth cleaning.

Results

In *Tab. 1* average clinical parameters values at the beginning and after 10 months from microsurgical periodontological treatment was shown. Average depth of the periodontal pockets was 8.5 mm and loss of the connective tissue attachment average about 2.2 mm was found. Rebuilding of bone of alveolar process was the effect of the treatment too (*Tab. 2*). The level of the bone estimated by x-ray pictures grow during 10 months average about 3.1 mm depth and 1.5 mm width of bone loss. Defect filling percentage is about 67.1%.

Discussion

Surgical treatment using biomaterials is an important part of comprehensive periodontal diseases cure. Results of the own research shows an advisability of using minor surgery regeneration. It causes a significant improvement of the clinical and radiological indexes. Our results are similar to the other authors [1-16]. Among our patients average starting values such as PPD and CAL were higher so the status of the patients was more advanced about 1.5-2.5 mm) from those which other researchers have noted [3,4,6-8,10-13,16].

Shallow of the periodontal pockets which we achieved was similar to the other authors and was about 2.9-3.6 mm [4,6,7]. Most of the reviews concern much bigger reduction of the depth of periodontal pockets (4.0 to 4.7 mm) [3,8,10,12,13,16]. Rebuilding of the connective tissue attachment which we found was lower average about 2.2 mm from most of reviews [3,8,10,12,13,16]. They concerned average values from 3.0 to 4.2 mm. Only a few researchers noted values similar to ours [4,6,7]. We should remember that CAL and PPD values are obtained faster than the rebuilding of the bone and the noted parameters during examination do not recognize the tissues but only give the picture of their resistance for pressure grow. That's why we need x-ray examination. After 10 months we noticed bone rebuilding with 67.1% cavity filling. This results are similar to those the other authors show. Parashis et al. [8] noted 61%, Heden [3] and Heil et al. [4] 70%. Using albumen of enamel is a great method of stimulation destructed periodontal pockets regeneration. We should expect that longer observation period will give better clinical parameters values.

Motions

Emdogain – albumen of enamel matrix is easy in use, safe and effective method to rebuilding of destructed periodontal structures.

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Activity of lysosomal exoglycosidases in saliva of patients with HIV infection

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Abstract

Introduction: The aim of this work was to evaluate the influence of HIV infection on the catabolism of glycoconjugates in oral cavity, by determination the activity of lysosomal exoglycosidases in resting whole saliva HIV positive patients.

Material and methods: Sample of resting whole saliva from HIV infected patients (divided into two groups, depending on lymphocyte CD4+ number in peripheral blood) and the control-HIV negative group were analyzed for exoglycosidases activity. Determinations the activities (μKat/kg of protein) of lysosomal exoglycosidases were performed according to Chatteriee et al., modified Zwierz et al. The protein content (mg/ml) was determined by the Lowry method. Statistical analysis was performed using packet Statistica 6.0. Results were expressed as the mean and SD. P values less than 0.05 were considered significant.

Results: Exoglycosidases activities were not statistically dependent on immunological status of HIV patients. We obtained insignificant increase activities of HEX, HEX A and GALβ and insignificant decrease activity of HEX B along with the reduction of the CD4+ number. In both HIV positive groups the activities of HEX B were statistically lower and GALβ statistically higher in comparison to the control. In the case of HEX A significant differences could be observed between patients with low immunological status and the control group.

Conclusions: HIV infection intensifies catabolism glycoconiugates in saliva and changes activities of HEX, its isoenzymes A and B and β-galactosidase. It may change susceptibility the cells lining oral cavity to viral and bacterial infections.

Key words: HIV, lymphocyte CD4+, human saliva, lysosomal exoglycosidases.

Introduction

Glycoconiugates (glycoproteins, glycolipids and proteoglycans), form membranes of cells covering oral cavity, membranes on the teeth and intercellular substance of gingival's connective tissue [1]. Degradation the sugar moieties of glycoconjugates is performed by aminohydrolases, endoglycosidases and lysosomal exoglycosidases [2,3]. In normal saliva, the activity of lysosomal exoglycosidases is small, but sufficient to maintain steady state of glycoconjugates metabolism [4]. HIV infection can coexist with peridontopathy and neoplasm in oral cavity [5].

Chronic inflammation is accompanied by accumulation of neutrophiles, lymphocytes and mastocytes, which take part in destruction of the soft tissue of the oral cavity. Activity of proteolytic enzymes and enzymes degrading the glycoconiugates intensifies inflammatory changes and dystrophic digestion the host tissues in paradontium, and stimulates growth of microorganisms which participate in patomechanism of paradontitis, e.g. *Actinobacillus actinomycetemcomitans*, *Capnocytophag* [6].

The saliva is used to immunological and biochemical diagnosis of diseases of paradontium [7] with regard on easy accessibility and the possibility of non-invasive taking. Therefore the aim of the present work was evaluation the influence of the HIV infection on the catabolism of glycoconjugates in oral cavity, by determination the activity of lysosomal exoglycosidases in saliva of HIV positive patients.

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***Table 1*. Activity of lysosomal exoglycosidases in saliva of HIV positive patients and the control group**

Group	N	isoenzyme B μKat/kg protein		isoenzyme A μKat/kg protein		hexosaminidase (HEX) μKat/kg protein		β-galaktozydase (Gal) μKat/kg protein	
		mean	SD	mean	SD	mean	SD	mean	SD
I CD4+>500	12	7.54	1.55	8.95	1.33	16.49	1.48	2.03	0.108
II CD4+<499	37	7.09	0.58	10.25	0.62	17.35	1.03	2.04	0.09
III control	32	12.06	1.08	7.13	0.49	19.19	1.27	1.61	0.05
I:III		0.0229						0.0056	
p II:III		0.000288		0.000261				0.000041	

Material and methods

Consent of 49 HIV infected and 32 healthy persons (control – III) was obtained in accordance with guidelines of the Ethics Committee of Medical University of Białystok who approved the study (grant nr 3-70950L). HIV positive patients were hospitalized in Infectious Disease Clinic of Medical University in Białystok. These patients were divided into two groups: the Ist group – 12 persons CD4+>500/ml (I), the IInd group – 37 persons CD4+<500/ml (II).

3 ml of unstimulated, whole saliva was collected on ice by spitting method, under standardized conditions [8].

Salivary samples were centrifuged at 3000 x g for 20 minutes at 4°C to remove cells and debris [8]. The resulting supernatant was divided on 200 μl portions, frozen and kept at -80°C until analyzed.

Check-up of oral cavity was done in artificial light by use diagnostic dental tools. Condition of gingivae was evaluated by gingival index (GI) determined by the method Löe and Silness and papilla bleeding index (PBI).

Determinations the activities (μKat/kg of protein) of HEX, HEX A, HEX B and GALβ were performed according to Chatteriee et al. [9] in modification [3]. To 30 μl of substrate (p-nitrophenyl-N-acetyl-β-glucosaminide or p-nitrofenyl-β-D--galaktopiranozyde, Sigma) and 40 μl of 0.1M phosphate-citrate buffer, pH 4.7 or 4,3, 10 μl of diluted supernatant were added. Incubation time was 60 minutes at 37°C and reaction was stopped by adding 200 μl of 200 mM borate buffer, pH 9.8. Isoenzyme HEX A activity was calculated as the difference between the total activity of the enzyme and HEX B activity. The liberated p-nitrophenol was measured spectrophotometrically in a microplate reader, the Elx800™ at 405 nm. The protein content in supernatants (mg/ml) were measured according to the method of Lowry with BSA (Sigma) as a standard [10]. All determinations were performed in duplicate.

ANOVA followed by NIR test, and Spearman correlation were used for the statistical analysis. Results were expressed as the mean and SD. A level of $p \leq 0.05$ was considered to be significant.

Results

Results of this experiment are summarized in *Tab. 1*. One could conclude that HEX B activity in saliva of HIV-infected patients was not dependent on their immunological status and there was statistically lower in comparison to the control group (I and III $p<0.02$: II and III $p<0.0002$).

HEX A activity of infected patients was negatively correlated with the amount of lymphocyte CD4+ in peripheral blood. However, the significant differences could be observed between patients with low immunological status and the control group ($p<0.0002$).

HEX activity of infected patients increased with the decrease of the lymphocyte CD4+ number and it showed tendency to be lower in the comparison to the control.

GALβ activity in saliva of HIV positive groups was not changed with HIV progression and was statistically higher than in the control group (I and III $p<0.005$; II and III $p<0.00004$).

Discussion

It was reported, that HIV infects sensibility cells by binding to the receptor CD 4, and in case of lymphocytes deprived of CD4, through other receptors, e.g. the mannosidic or galactosidic type. The major determinant of viral tropism is at the level of entry. This occurs only if the appropriate coreceptor is present. Entry of HIV-I into its CD4+ target cells requires fusion/entry cofactors. Recently, the seven-transmembrane, G protein-coupled chemokine receptors CXCR4 and CCR5 were identified as cofactors for fusion and entry of T cell (T)3-tropic and macrophage (M)-tropic strains of HIV-1, respectively, into CD4+ cells [11,12]. CCR5 is the major coreceptor for HIV transmission *in vivo*. Except enzymes being part of innate immunity (lactoferrin, lysozyme, salivary peroxidase), in literature we did not find any data on influence of HIV infection on enzymes in saliva [13].

The aim of the present work was evaluation the activity of lysosomal exoglycosidases in saliva of HIV patients as indicators of glycoconjugates catabolism. Exoglycosidases [4] together with

aminohydrolases and endoglycosidases take part in degradation of glycoconjugates [2]. The glycoconjugates (glycoproteins, proteoglycans and glycolipids) are receptors, or transporters [14] on surface of cellular membranes. Catabolism of glycoconjugates is connected with maintaining balance between degradation of old and synthesis new molecules.

We estimated activity of N-acetyl-β-hexosaminidase, its isoenzymes (thermolabile isoenzyme A and themostabile isoenzyme B) and β-galactosidase in saliva of patients infected with HIV. The obtained results were analyzed depending on peripheral blood lymphocyte CD^{4+} level. Exoglycosidases activities were not statistically dependent on immunological status of HIV positive patients. We only could observed insignificant increase activities of HEX, HEX A and GAL β and insignificant decrease activity of HEX B along with the reduction of the CD^{4+} amount. Additionally in both HIV positive groups the activities of HEX B were statistically lower and GAL β statistically higher in comparison to the control. In the case of HEX A significant differences could be observed between patients with low immunological status and the control group. The lack of information in the literature on activity of exoglycosidases in saliva of HIV infected patients, did not permit on comparison our results with data of other authors. It was reported that lymphocytes and macrophages are source of lysosomal exoglycosidases in saliva [15]. There is unknown mechanism the influence of HIV infection on activity of exoglycosidases and influence the activity of exoglycosidases on HIV infection. It is known, that receptors for HIV are glycoproteins, but it is unknown if, and what part of oligosaccharide chains on HIV envelope binds to receptor on surface of sensitive cells. It should be supposed, that exoglycosidases removing appropriate sugars from non reducing end of oligosaccharide chains, can modify the possibility and strength of binding the envelope of HIV to cellular receptors, by the exposure of suitable oligosaccharide structures on surface of sensitive cells. Thus the exoglycosidases can influence the docking HIV to the cell receptor.

HIV infection is associated with enhanced apoptosis in CD4 T cells infected by HIV and in uninfected T cells. Death of the cell by apoptosis or necrosis is preceded be the damage of cellular membranes, and lysosomal included, and liberation their content. Damage to lysosomal membranes of salivary glands may increase liberation of exoglycosidases to saliva and change their activities. Release the content of lysosomal granules to the extracellular matrix and saliva is responsible for inflammatory state in oral cavity associated with HIV infection [6]. The observed by us changes in activity of lysosomal exoglycosidases in saliva of infected patients may result from: the mutations the sequences of DNA coding lysosomal exoglycosidases, the disorders in biosynthesis the polypeptide chains for lysosomal exoglycosidases, influence of virus on chaperones, changes in activity glycosylotransferases damage by HIV membranes of endoplasmatic reticulum and Golgi apparatus, which synthesize the oligosaccharic chains of lysosomal exoglycosidases, disturbances of intracellular transport of exoglycosidases, by influence on Man-6-P receptors or GGA proteins. This later hypothesis is particularly interesting because HIV has affinity toward mannosidic receptor. HIV binding to the mannosidic receptor may block binding Man-6-P of the oligosaccharide chain of lysosomal exoglycosidases with their receptor, and it stops exoglycosidase in trans Golgi compartment, or export outside the cell with omission of lysosomes.

Conclusions

Infection by HIV intensifies catabolism glycoconiugates in saliva and changes the activities of HEX, its isoenzymes A and B and β-galactosidase. It can be a reason of changes in susceptibility the cells lining oral cavity to viral and bacterial infections.

Abbreviation

HEX-N – acetyl-β-hexosaminidase
HEX A – isoenzyme A of N-acetyl-β-hexosaminidase
HEX B – isoenzyme B of N-acetyl-β-hexosaminidase
GAL β – β-galactosidase

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Incidence rate of *Candida* species in the oral cavity of middle-aged and elderly subjects

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Abstract

Purpose: The aim of this study was to determine the incidence rate of oral *Candida* species in middle-aged and elderly subjects.

Material and methods: The study carried out in 103 adults aged 35-92 years, in which 32 (31.1%) used complet or partial acrylic dentures. Mycological tests were performed by using culture (Sabouraud agar) and API 20C AUX (bioMérieux) for identification of the species level. Material for analysis included swabs taken from the palate mucosa and mucosal part of denture surfaces in denture wearers, as well as, from tooth surface and/or dentine carious lesions. The dental caries status of each patients was evaluated using DMF index (WHO 1986 criteria).

Results: Yeasts of *Candida* genus were isolated in 65/103 (63.1%) adults. The incidence rate of *Candida* spp. was higher in adults without dentures (46/71; 64.8%) compared to denture wearers (19/32; 59.4%); however, the differences were not statistically significant ($p=0.59 > p=0.05$). *Candida albicans* were the most frequently isolated species, and with a comparable rate ($p=0.06$), both in adults with and without dentures (17/32; 53.1% and 38/71; 53.5%, respectively). In 3 individuals without dentures, two other species were found apart from *C. albicans*, namely *C. glabrata* (2x) and *C. krusei* (1x). In a total of 11/49 (22.5%) strains belonging to 5 non- *C. albicans* species were detected in adults without dentures, while in denture wearers only 2/19 (10.5%) other species were found (*C. krusei* and *C. oralis*) ($p=0.26 > p=0.05$). Strains of *C. glabrata* species were isolated only from the elderly. No significant differences were noted in the incidence of *Candida* spp. between middle-aged subjects (35-44 years) (35/52; 67.3%) and the elderly (>55 years) (30/51; 58.8%) ($p>0.05$), both in denture wearers and non-denture wearing subjects. However, the frequency of oral *Candida* spp. strains was increased in advanced age subgroup 71-92 years (74.2%) compared with 56-70 years (35.0%) of elderly subjects ($p<0.05$), only in denture wearers (30.0% vs 5.0%) ($p<0.05$). The sex and DMF index distribution of both subject groups had no significant influence on the numbers of *Candida* spp. detected.

Conclusions: Yeasts of the genus *Candida* were isolated at a comparable rate ($p>0.05$) from the oral cavity of adults with and without dentures, as well as in middle-aged (35--44 years) and elderly subjects (56-92 years). However, a significant difference was observed only between elderly subgroups aged 56-70 (35%) and advanced age subgroup 71-92 years (74%).

Key words: adult subjects, denture wearers, oral *Candida albicans*, non-*C. albicans* species, DMF index.

Introduction

Candida species are ubiquitous yeasts and common residents of mucosal surfaces of the human oral cavity, the gastrointestinal and the urogenital tract [1-4]. Essentially all areas of the human gastrointestinal tract can harbor *Candida*. The most commonly isolated species (50 to 70% of yeast isolates) from the human gastrointestinal tract is *Candida albicans*, followed by *C. tropicalis*, *C. parapsilosis*, and *C. glabrata* [3] .

Candida spp. can be present in clinical specimens as a result of environmental contamination, colonization, or actual disease processes. An accurate diagnosis requires proper handling of clinical material. *Candida* spp. that are members of the normal microbiota with high prevalence in the normal population, can invade tissue and cause oral candidosis (candidiasis) or

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Table 1. **Isolation frequency (%) of oral *Candida* spp. in middle-aged and elderly subjects without dentures**

	35-44 years n=41	>55 years n=30	Total n=71
C. albicans	21 (51.2)	14 (46.7)	35 (49.3)
C. albicans + *C. glabrata*		2 (6.7)	2 (2.8)
C. albicans + *C. krusei*	1 (2.4)		1 (1.4)
C. glabrata		2 (6.7)	2 (2.8)
C. krusei		1 (3.3)	1 (1.4)
C. lusitaniae	2 (4.9)		2 (2.8)
C. pelliculosa	1 (2.4)		1 (1.4)
C. pulcherrima	2 (4.9)		2 (2.8)
Candida spp.	27 (65.8)	19 (63.3)	46 (64.8)

Table 2. **Isolation frequency (%) of oral *Candida* spp. in middle-aged and elderly subjects with dentures**

	35-44 years n=11	>55 years n=21	Total n=32
C. albicans	7 (63.6)	10 (47.6)	17 (53.1)
C. krusei		1 (4.8)	1 (3.1)
C. oralis	1 (9.1)		1 (3.1)
Candida spp.	8 (72.7)	11 (52.3)	19 (59.4)

life-threatening disease in patients whose immune defenses have been altered by old age, disease or iatrogenic intervention [1-6]. Mucocutaneous forms of candidiasis are often related to defects in cell-mediated immunity, while systemic spread is generally associated with neutropenia [2,4-7].

As a result of increasing numbers of immunocompromised individuals within the human population, the incidence of *Candida* infections has increased dramatically in the last decade [4-6].

Among species of the genus *Candida*, *C. albicans* is the prevalent causative agent of candidiasis and constitutes the fourth most common nosocomial bloodstream isolate in industrial countries [4,6]. It is generally belived that candidiasis arises from endogenous commensal strains inhabiting the oral cavity, gastrointestinal tract and genitourinary system [3,4,7-9].

Cannon et al. [9] have proposed that *Candida* colonization of oral surfaces, including the denture-fitting surface, can serve as a reservoir for disseminated infections such as aspirate pneumonia and gastrointestinal infections.

The aim of this study was to determine the incidence rate of oral *Candida* species in middle-aged and elderly subjects.

Material and methods

The study carried out in 103 adults, aged 32-92 years, in which 32 (31.1%) used complet or partial acrylic dentures.

Dental epidemiological examinations of the patients from Białystok, and the surroundings carried out in two groups: 1. From dental outpatients clinic 52 patients (35 female and 17 male) aged 35-44 years (middle-aged), 2. Patients from the Geriatric Social Center of Białystok, 51 residence (27 female and 24 male), older than 55 years (56-92 years) (elderly subjects).

History and clinical examinations done using a probe, a mirror, and a WHO periodontometer. Data was recorded on special WHO card (Oral Health Assessment form 1986) used for dental epidemiological assessment.

Dental status evaluated by using of mean value calculated by DMF index, which indicate the severity of caries (D= number of teeth with decay, M= number of missing teeth, F= number of filling teeth).

Mycological tests were performed in all the patients and included culture (Sabouraud agar) and identification to the species level (API 20C AUX; bioMérieux) [4]. The swabs taken from the palate mucosa and mucosal part of denture surfaces in denture wearers, as well as from tooth surface and/or dentine carious were analysed.

The local ethics committee approved this study, and all subjects gave informed consent to the procedures.

The statistical analysis was done using the chi-square test ($p \leq 0.05$).

Results

Yeasts of *Candida* genus were isolated in 65/103 (63.1%) adults. Among them *Candida albicans* species predominate (52/65; 80.0%) ($p=0.0001$). The incidence rate of *Candida* spp. was higher in adults without dentures (46/71; 64.8%) (*Tab. 1*) than with denture (19/32; 59.4%) (*Tab. 2*); however, the differences were not statistically significant ($p=0.59>p=0.05$).

Candida albicans were the most frequently isolated species, and with a comparable rate ($p=0.06$), both in adults with and without dentures (17/32; 53.1% and 38/71; 53.5%, respectively) (*Tab. 1* and *2*). In 3 individuals without dentures, two other species were found apart from *C. albicans*, namely *C. glabrata* (2x) and *C. krusei* (1x) (*Tab. 1*). A total of 11/49 (22.5%) strains belonging to 5 non-*C. albicans* species were detected in adults

***Table 3*. Isolation frequency (%) of oral *Candida* spp. in elderly subjects with and without dentures**

	56-70 years			71-92 years		
	Denture n=8	No-denture n=12	Total n=20	Denture n=13	No-denture n=18	Total n=31
C. albicans	1 (12.5)	3 (25.0)	4 (20.0)	9 (69.2)	11 (61.1)	20 (64.5)
C. albicans + *C.glabrata*		1 (8.3)	1 (5.0)		1 (5.6)	1 (3.2)
C. glabrata		1 (8.3)	1 (5.0)		1 (5.6)	1 (3.2)
C. krusei		1 (8.3)	1 (5.0)	1 (7.7)		1 (3.2)
Candida spp.	1 (12.5)	6 (25.0)	7 (35.0)	10 (76.9)	13 (72.2)	23 (74.2)

without dentures, while only 2/19 (10.5%) other species were found (*C. krusei* and *C. oralis*) in denture wearers (*Tab. 2*) p=0.26 >p=0.05). Strains of *C. glabrata* species were isolated only from the elderly (>55 years) (*Tab. 1*). No significant differences were noted in the incidence of *Candida* spp. between middle-aged subjects (35-44 years) (35/52; 67.3%) and the elderly (>55 years) (30/51; 58.8%) (p>0.05), both in denture wearers (*Tab. 1*) (p=0.826) and non-denture wearing subjects (*Tab. 2*) (p=0.515). However, a significant difference was observed only between elderly subgroups aged 56-70 and 71-92 years old (*Tab. 3*).

The isolation frequency of oral *Candida* spp. strains was more in the advanced age subgroup 71-92 years (23/31; 74.2%) compared with elderly subjects aged 56-70 years (7/20; 35.0%) (p=0.0034); seen only in denture wearers (10/13; 76.9% and 1/8; 12.5%, respectively), and it was statistically significant (p=0.0155). Among elderly patients aged 56-70 years, *Candida* spp. was most often isolated from patients without dentures (6/20; 30.0% vs 1/20; 5%) (p=0.0375) (*Tab. 3*).

The dental caries severity in adult aged 35-44 years detected by using DMF index was very high, about 21.4; and was higher in 35 females (22.9) than in 17 males (18.25). A major influence on the DMF value in this age group was the number of filling teeth, and F component was 9.9 in whole group (11.5 filling teeth in female and only 6.4 in male). In this age group observed more healthy teeth in male (11.6 healthy teeth) than in female (7.3).

The mean value of DMF for the elderly subject (55-92 years) from the second group was 30.5, and was comparable in 24 male (30.7) and 27 female (30.2). This high value was due to numbers of missing teeth (M), with mean value 26.4 teeth (27.1 in male and 25.6 in female). In this group we observed a few number of filling teeth (mean F=0.2; in female =0.1, in male 0.2), and very few number of teeth present in oral cavity (mean 5.7 teeth, where only 1.5 was healthy). More teeth were in male (mean =6.3) than female (mean =5.1), however, more healthy teeth were seen in female (mean =1.7) compared to male.

The mucous membrane changes of oral cavity were seen in 41(80.4%) elderly subjects (18 female and 23 male). Xerostomia more frequently detected in female (44.4%), while tongue disorders in male (60.9%).

The sex and DMF index distribution of both age groups 35-44 and 56-92 years had no significant influence on the numbers of *Candida* spp. detected.

Discussion

It has generally been assumed that old age represent a predisposing condition for increased candidal colonization. Lockhart et al. [10] demonstrated that frequency and intensity of carriage of candidal colonization increased as a function of age, independent of denture use. However, Ikebe et al. [11] showed that candidal activity was not significantly associated with age or gender in the relatively healthy people. The activity of *Candida* species in the oral cavity was associated with the wearing of removable dentures and stimulated salivary flow, independent of age or gender even in the relatively healthy elderly (mean age of 66.7±4.3 – s.d. – years) [11].

Commensal existence of intraoral *Candida* species varies from 20 to 50% in a healthy edentulous population [12,13] and up to 75% in a population wearing dentures [13,14].

Our previous studies have shown a high incidence of isolation of *Candida* species from oral cavities of healthy middle-aged (about 40 years) denture wearers (75%) and healthy people with their own dentition (41%) [15].

According to our results presented here, the prevalence rate of *Candida* spp. in the oral cavity of adults with (59.4%) and without dentures (64.8%) (p>0.05), as well as in middle-aged (35-44 years) and elderly subjects (56-92 years) was high (67% vs 59%) with comparable rate (p>0.05). However, the frequency of oral *Candida* spp. strains was increased in advanced age subgroup 71-92 years (74.2%) compared with 56-70 years (35.0%) of elderly subjects (p<0.05), only seen in denture wearers (30.0% vs 5.0%) (p<0.05).

Belazi et al. [16] also did not reveal any association between age and *Candida* growth in any of the study groups such as the diabetic patients and the group of healthy subjects with a mean age of 54±7 years (range 40-80 years). Although the results showed an equal prevalence of candidal growth among healthy and diabetic adults wearing dentures. For older age (>60 years old) when combined and presence of dentures, a statistically significantly greater proportion of subjects with diabetes mellitus suffered from candidiasis [16].

We speculate, similar to Belazi et al. [16], that the oral carriage of *Candida* spp. cannot be directly associated with either age or presence of dentures. Certain systemic conditions (e.g. diabetes mellitus), defects in the immune system [4,5,7,10], and/or some medications (e.g. antibiotics, corticosteroids) may predispose the transformation of a benign colonization, such as *Candida* species, into opportunistic pathogens [19]. Denture use

and hyposalivation are common not only in the frail elderly, but also in the comparatively healthy elderly who live independently [11,20]. The Japanese national survey of dental diseases by the Ministry of Health and Welfare collected data from every member of all households in 300 municipalities that had been randomly sampled in every prefecture [11]. The survey found that the number of residual teeth gradually declined after age 50. Consequently, 50% of people between 65 and 74 years and 70% of those between 75 and 84 used removable dentures.

Tokajuk et al. [21] evaluated prevalence of toothlessness in 591 subjects of geriatric population of the north-eastern of Poland and found that 37.0% of them were edentulous. The study supervised by WHO, indicate that there is a need for prosthetic treatment in elderly subjects, especially in older age groups (65-74 years and ≥75 years old) and who lived in institutions. Stokowska et al. [22] examined the middle-aged adults (35-44 years) living in Białystok during epidemiological studies carried out in 1987 and 1995. High mean DMF values were found in both 1987 (18.14) and 1995 (17.24). These values were higher than for all Polish population (18.6 and 16.5, respectively).

Our study showed that DMF indices both in middle-aged adults (35-44 years) (21.4) and in elderly subjects (55-92 years) (30.5) were very high. However, DMF index and sex distribution of both subject groups had no significant influence on the number of *Candida* species detected.

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Microorganisms in root carious lesions in adults

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Abstract

Purpose: Root caries is emerging as a significant problem in the middle aged and elderly subjects because of the improving general health conditions, and medical and technological advances. The purpose of this investigation was to assess the prevalence of aerobic and anaerobic bacteria as well as yeasts of *Candida* genus in root carious lesions in middle-aged and older adults.

Material and methods: Specimens of root carious lesions were collected from 78 adults for bacteriological and mycological studies. Standard procedures of culture, isolation, and identification of aerobic and anaerobic bacteria, and fungi were used in the study.

Results: The analysis of results was performed independently in two age groups of adults, i.e. 52 subjects aged 35-44 years (middle age) and 26-aged 55-72 years (older age). There were 120 bacterial strains isolated from root carious lesions in middle-aged subjects, 63 (52.5%) strains belonged to 5 genera of aerobic bacteria and 57 (47.5%) – to 7 genera of anaerobic bacteria ($p>0.05$). While in the second group, 85 strains were isolated, 54 (63.5%) – 6 genera of aerobic bacteria and 31 (36.5%) – 4 genera of anaerobic bacteria ($p=0.0004$). There were no differences between the isolation rate of a various species in both examined groups, except for *Streptococcus* spp., *S. oralis*, *Micrococcus* spp., *Neisseria* spp. and *Veillonella* spp., which statistically significantly most frequent occurred in elderly ($p<0.05$). The yeasts of *Candida* genus of 4 species (*C. albicans*, *C. lusitaniae*, *C. pelliculosa*, and *C. pulcherrima*) were isolated from middle-aged subjects (32.7%) with the comparable rate to older adults (30.8%; only *C. albicans*) ($p>0.05$). Among all isolated microorganisms, *Candida* spp., were comprised about 10% in both examined groups ($p>0.05$).

Conclusions: Aerobic Gram-positive cocci (*Staphylococcus* spp. and *Streptococcus* spp.) as well as anaerobic ones (*Peptostreptococcus* spp.), and *Candida albicans* were occurred most frequently in root carious lesions in middle--aged and older adults.

Key words: root caries, middle-aged subjects, older adults, aerobic/anaerobic bacteria, *Candid*a spp.

Introduction

Root surface caries, as the name implies, occurs on root cementum or dentine and is caused by a microbial biofilm. The disease is secondary to gingival recession, since, in a healthy mouth, cementum and dentine are not exposed to the microflora and, therefore, are non expose for colonization [1-3]. Gingival recession can be caused by a number of factors, including old age (the most common factor), mechanical injury (excessive tooth brushing) or periodontal treatment regimens [3]. In developed countries proportion of the population over 65 years of age is increasing, additionally, the percentage of these remaining dentate is also increasing. A survey carried out by Steele et al. [4] in 1991-92 showed, amongs other things, that in Southern England 67% of patients over 60 years were dentate compared with equivalent cohort in 1962, where only 15% remained dentate.

The aetiology of root caries is multifactorial of which microbiological factor plays a critical role [1-3]. The microbiological nature of the associated plaque biofilm is different from that associated with crown caries (supragingival plaque) even though it is technically still a supragingival plaque [3]. The microbiology of this biofilm has been the subjects of numerous investigastions over the years, however, only recently have the problems associated with sampling of the infected underlying dentine been

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identified and addressed [5-7]. While there is ample evidence to imply a strong association between mutans group streptococci and coronal caries [1-3,5,6], similar data on microbiological agents in root caries is poorly understood.

Some epidemiological studies have shown that *Actinomyces* spp. are predominant bacteria in root caries [1-3,6-9], while other have demonstrated a variety of bacteria and failed to implicate any single bacterial genus as a predominant isolate [3,6,9]. As no data are available on the microbiota of root caries lesions in Polish population, the main aim of study was to evaluate qualitatively the microbiology of root caries in middle- aged and older adults living in Białystok.

Material and methods

Samples of root carious lesions from a total 78 subjects (51 females and 27 males; age range: 35-72 years), provided by dentists practicing in the Białystok, were examined. Among them, 52 (66.7%) adults were classified to middle-aged group 35-44 years (mean 39.6 years; 35 females and 17 males) and 26 (33.3%) to older group 55-72 years (mean 64.3 years; 16 females and 10 males). Samples were taken from selected teeth of subjects as parts of ongoing dental therapy and transferred to a transport medium. All samples were processed within 2 hours of sampling. Transport tubes were incubated in 37°C for 15 minutes and vigorously mixed for 20 to 30 seconds using a vortex mixer. Dilution aliquots of 100 μL were distributed onto two Brucella blood agar plates; 1 plate was used for aerobic incubation for 2 to 5 days at 37°C and the other plate was used for anaerobic incubation using Gas Pak system (Becton-Dickinson) for 2 to 7 days at 37°C. Selective media such as McConkey, Chapman, Cetrimide, Rogosa agar (for *Lactobacillus* spp. and *Bifidobacterium* spp.) and Sabouraud glucose agar for yeasts (*Candida* spp.) were also inoculated.

Charcoaled paper points, as well as the remaining fluid, were transported to a semiliquid medium for anaerobic bacteria and incubated at 37°C for up to 14 days. In cases of no growth on the agar plates, tubes were checked daily for turbidity during 14 days.

Bacteria growing on agar plates were preliminarily identified based on colony morphology, Gram stain and oxygen tolerance. Isolates were further identified into genus and/or species based on selective media and API system (API Staph, API Strep, API NH, API Coryne, API 20E, API 20 A, API 20 NE and API 20C AUX) (bioMérieux) [10,11].

This study was approved by the Bioethics Committee of the Medical University of Białystok. Informed consent was obtained from all participants.

The isolation frequency of microorganisms in the different groups of examined subjects were analysed with the Chi-squared test.

Results

The analysis of results was performed independently in two age-groups of examined adults, i.e. 52 (66.7%) subjects aged 35-44 years (middle age) and 26 (33.3%) – aged 55-72 years (older age) (*Tab. 1*). There were 120 bacterial strains isolated from root carious lesions in middle-aged subjects, 63 (52.5%) strains belonged to 5 genera of aerobic bacteria and 57 (47.5%) – to genera of anaerobic bacteria ($p>0.05$). While in the second group, 85 strains were isolated, 54 (63.5%) – 6 genera of aerobic bacteria and 31 (36.5%) – 4 genera of anaerobic bacteria ($p=0.0004$) (*Tab. 1*).

Among all isolated microorganisms, *Candida* spp. were compromised about 10% in both examined groups (17/137; 12.4% vs 8/93; 8.6%) ($p=0.3627$) (*Tab. 1*).

There were no differences between the isolation rate of a various species or genera in both examined groups, except for *Streptococcus* spp. (12/52; 23.1% vs 19/26; 73.1%) ($p=0.001$), *Streptococcus oralis* (1/52; 1.9% vs 7/26; 26.9%) ($p=0.0006$), *Micrococcus* spp. (0 vs 2/26; 7.7%) ($p=0.04$), *Neisseria* spp. (7/52; 17.3% vs 12/26; 46.2%) ($p=0.007$) and *Veillonella* sp. (8/52; 15.4% vs 9/26; 34.6%) ($p=0.053$), which statistically significantly most frequently occurred in elderly (*Tab. 2*).

Table 1. **Number of bacterial and fungal strains isolated from root carious lesions in adult patients**

Microorganisms	35-44 years (n=52)	55-72 years (n=26)	Total (n=78)
I. Aerobic bacteria	**63**	**54**	**117**
1. Gram-positive	49	39	88
Gram-positive cocci	49	39	88
Streptococcus spp.	12	19	31
Gemella morbillorum	2	-	2
Staphylococcus spp.	35	18	53
Micrococcus spp.	-	2	2
2. Gram-negative	14	15	29
Gram-negative cocci	9	12	21
Neisseria spp.	9	12	21
Gram-negative rods	5	3	8
Haemophilus parainfluenzae	5	2	7
Escherichia coli	-	1	1
II. Anaerobic bacteria	**57**	**31**	**88**
1. Gram-positive	45	22	67
Gram-positive cocci	40	19	59
Peptococcus spp.	6	-	6
Peptostreptococcus spp.	34	19	53
Gram-positive rods	5	3	8
Actinomyces spp.	3	2	5
Bifidobacterium spp.	-	1	1
Lactobacillus spp.	1	-	1
Propionibacterium spp.	1	-	1
2. Gram-negative	12	9	21
Gram-negative cocci	11	9	20
Veillonella spp.	11	9	20
Gram-negative rods	1	-	1
Bacteroides caccae	1	-	1
Bacteria:	120	85	205
Gram-positive	94	61	155
Gram-negative	26	24	50
III. *Candida* spp.	**17**	**8**	**25**
Total	**137**	**93**	**230**

***Table 2*. Isolation frequency (%) of microorganisms from root carious lesions in adult patient according to age**

Microorganisms	35-44 years (n=52)	55-72 years (n=26)	Total (n=78)
Streptococcus spp.	*23.1	*73.1	39.7
S. intermedius	1.9	3.8	2.6
S. mitis	5.8	15.4	9.0
S. oralis	*1.9	*26.9	10.3
S. salivarius	3.8	7.7	5.1
S. sanguis	3.8	3.8	3.8
S.vestibularis	5.8	15.4	9.0
Gemella morbillorum	3.8	0	2.6
Staphylococcus spp.	67.3	69.2	67.9
S. aureus	3.8	3.8	3.8
S. caprae	1.9	0	1.3
S. capitis	3.8	7.7	5.1
S. epidermidis	34.6	42.3	37.2
S. haemolyticus	1.9	0	1.3
S. hominis	3.8	7.7	5.1
S. saccharolyticus	1.9	0	1.3
S. simulans	7.7	3.8	6.4
S. warneri	7.7	3.8	6.4
Micrococcus spp.	0	*7.7	2.6
Neisseria spp.	*17.3	*46.2	26.9
N. flavescens	3.8	7.7	5.1
N. mucosa	1.9	7.7	3.8
N. sicca	3.8	11.5	6.4
N. subflava	7.7	19.2	11.5
Haemophilus parainfluenzae	9.6	7.7	9.0
Escherichia coli	0	3.8	6.4
Peptococcus spp.	11.5	0	7.7
Peptostreptococcus spp.	65.4	73.1	67.9
Bifidobacterium spp.	0	3.8	1.3
Lactobacillus spp.	1.9	0	1.3
Propionibacterium spp.	1.9	0	1.3
Actinomyces spp.	5.8	7.7	6.4
A. naeslundii	3.8	7.7	5.1
Actinomyces sp.	1.9	0	1.3
Veillonella spp.	21.2	34.6	25.6
V. parvula	5.8	0	3.8
Veillonella sp.	*15.4	*34.6	21.8
Bacteroides caccae	1.9	0	1.3
Candida spp.	32.7	30.8	32.1
C. albicans	26.9	30.8	28.2
C. lusitaniae	1.9	0	1.3
C. pelliculosa	1.9	0	1.3
C. pulcherrima	1.9	0	1.3

* $p<0.05$

The yeast of *Candida* genus of 4 species (*C. albicans*, *C. lusitaniae*, *C. pelliculosa*, and *C. pulcherrima*) were isolated from middle-aged subjects (17/52; 32.7%) a comparable rate to older adults (8/26; 30.8%; only *C. albicans*) (p=0.8638) (*Tab. 2*).

Discussion

It is thought that the root carious lesion occurs as a function of accumulation and subsequent stagnation of a plaque biofilm at the gingival margin [3]. The nature of the microbiota is poorly understood, but there seems to be no single species responsible for disease and progression [3,6,9]. What may be important is the presence of particular strains of a defined but heterogenous group of bacteria that are particularly suited to that environment [3]. Indeed, Sansone et al. [9] have shown that the acidogenic and aciduric flora associated with a carious lesion is far more diverse (approximately 25 taxa) than the corresponding acidogenic and aciduric flora associated with sound root surfaces (approximately eight taxa).

Beighton and Lynch [5] showed that the bacterial composition of the carious dentine biofilm associated with "soft" lesions consists of significantly more lactobacilli and Gram-positive pleomorphic rods and conversely, significantly fewer streptococci compared with the overlying plaque biofilm. Additionally, there is an increased number and/or proportion of *S. mutans* in "soft" lesions compared with "hard" lesions or sound surfaces. The lesion has been shown to have a definite progression, since changes in its clinical appearance are observed over time [3,5].

Actinomyces spp. have historically been associated with root surface caries, although the nomenclature of the species and genospecies in the literature confuses the matter greatly; see Johnson et al. [7]. However, in recent studies by Brailsford et al. [8,12] *Actinomyces naeslundii* was shown not to be associated with active carious lesions and that *A. israelii* and *A. gerencseriae* predominated. Shen et al. [13] also isolated three different *Actinomyces species*, including *A. israelii*, *A. meyeri* and *A. odontolyticus*, amongst which *A. israelii* was most predominant. The predominant microorganisms isolated by Shen et al. [13] from 30 root caries lesions were *Lactobacillus* spp. (90%), *Streptococcus* spp. (100%) and *Actinomyces* spp. (63%). This result of the present Chinese subject was in agreement with studies, most of which conducted on Caucasians subjects, at other parts of the world [14,15]. More recent studies on root carious lesions also indicated that predominant cultivable flora were streptococci, lactobacilli, *Actinomyces* spp. and staphylococci [16,17].

In the present study, we isolated only 5 (6.4%) strains of *Actinomyces* spp. from 78 carious lesions of adult subjects. Among them, 4 strains belonged to one species of *Actinomyces naeslundii*. This species was absence in 30 such lesions in elderly institutionalized, ethnic Chinese [13]. From our subject examined also not frequently was isolated *Lactobacillus* spp. (only one strain). The predominant microorganisms isolated from root carious lesions were *Streptococcus* spp. and *Peptostreptococcus* spp. (73.1% each) in older adults, while *Staphylococcus* spp. and *Peptostreptococcus* spp., as well as in middle-aged (67.3% and 65.4%, respectively) and overall subjects studied (67.9% each genus).

Shen et al. [13] isolated from root caries of elderly population also *Staphylococcus* spp. and *Peptostreptococcus* spp., and other taxa such as *Veillonella* spp. and *Candida* spp. The isolation frequency of *Veillonella* spp. in our study was 25.6%, and was comparable between older (34.6%) and middle-aged (21.2%) subjects (p=0.1993). The isolation frequencies of *Veillonella* spp. in root caries represented by different researches vary. In two reports Ellen et al. [14] and van Houte et al. [16] recorded 96% and 42-100% isolation frequency of *Veillonella* spp., respectively, from root caries. On the contrary, in our study

(25.6%) and in study performed by Shen et al. [13] (26.7%), was relatively low. From the host point of view, *Veillonella* spp. are considered beneficial organisms in the carious process as they metabolize short-chain carboxylic acids produced by neighbouring cariogenic flora [1,2].

The role of staphylococci in caries initiation and development is not well known. The recent study by Schupbach et al. [6] reported the proportions of staphylococci in initial and advanced lesions as 4.4 and 15.5%, respectively. In our study, 25.9% of the total bacterial isolates were identified as *Staphylococcus* spp.

Peptostreptococcus spp. are member of the normal oral microbiota, but some *Peptostreptococcus* species are thought to be associated with anaerobic infections including gingivitis and periodontitis [18].

In previous cohort studies of middle-aged healthy adults living in Białystok we have demonstrated, in general, a 41% oral carriage rate for yeasts [19]. The high isolation frequency (32.1%) of *Candida* spp. from root caries lesions of adult subjects in our study may suggeste that carious foci are reservoirs of yeasts.

There were no significant differences in root caries microbiology between denture wearers (22: 11 middle-aged and 11- older adults) and non-denture wearing subjects (56: 41 middle-aged and 15-old-age) (data not shown). These differences were observed by Shen et al. [13]. Notably, significantly more Gram-positive cocci, Gram-negative rods, *Staphylococcus* spp. *Actinomyces* spp. and *Candida* spp. ($p<0.05$) could be isolated from the non-denture wearing group [13].

In conclusion, the present study provides baseline information on the microbiologic features of root caries in the middle--aged and elderly subjects in the north-eastern region of Poland.

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