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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Received 21.03.2006 Accepted 30.03.2006

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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