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

Kowalski J*, Górska R, Dragan M, Kozak I

Department of Periodontology and Oral Diseases, Medical University of Warsaw, Poland

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.

* CORRESPONDING AUTHOR:
Department of Periodontology and Oral Diseases
Medical University of Warsaw
ul. Miodowa 18, 00-246 Warsaw, Poland
Tel./fax: +48 022 831 21 36
Skype: jankowalski1466
e-mail: jan_kowalski@o2.pl (Jan Kowalski)

Received 13.03.2006 Accepted 30.03.2006

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.

Acknowledgements

Authors would like to thank Pierre Fabre Medicament Polska, Poland and Hain Lifescience GmbH, Germany, for their kind support of the present study.

The study is financed by the Medical University of Warsaw (01-1S14/WE/2004).

References

1. Genco RJ. Current view on risk factors for periodontal disease. J Periodontol 1996; 67: 1041-9.
2. Kornman KS, Crane A, Wang HY, diGiovine FS, Newman MG, Pirk FW, Wilson TG Jr, Higginbottom FL, Duff GW. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997; 24: 72-7.
3. Faizuddin M, Bharathi SH, Rohini NV. Estimation of interleukin-1beta levels in the gingival crevicular fluid in health and in inflammatory periodontal disease. J Periodontal Res 2003; 38: 111-4.
4. Meisel P, Siegemund A, Dombrowa S, Sawaf H, Fanghaenel J, Kocher T. Smoking and polymorphisms of the interleukin-1 gene cluster (IL-1alpha, IL-1beta and IL-1RN) in patients with periodontal disease. J Periodontol 2001; 73: 27-32.
5. Meisel P, Siegemund A, Grimm R, Herrmann FH, John U, Schwahn C, Kocher T. The Interleukin-1 polymorphism, smoking, and the risk of periodontal disease in the population-based SHIP study. J Dent Res 2003; 82: 189-93.
6. Persson GR, Matuliene G, Ramseier CA, Persson RE, Tonetti MS, Lang NP. Influence of interleukin-1 gene polymorphism on the outcome of supportive periodontal therapy explored by a multi-factorial periodontal risk assessment model (PRA). Oral Health Prev Dent 2003; 1: 17-27.
7. di Giovine FS, Cork MJ, Crane A et al. Novel genetic association of an IL-1B gene variation a +3953 with IL-1B protein production and psoriasis. Cytokine 1997; 7: 606 (Abstract #A65).
8. Riviere GR, Smith KS, Tzagaroulaki E, Kay SL, Zhu X, DeRouen TA, Adams DF. Periodontal status and detection frequency of bacteria at sites of periodontal health and gingivitis. J Periodontol 1996; 67: 109-15.
9. Papapanou PN. Epidemiology of periodontal diseases: an update. J Int Acad Periodontol 1999; 1: 110-6.
10. Harper-Owen R, Dymock D, Booth V, Weightman AJ, Wade WG. Detection of unculturable bacteria in periodontal health and disease by PCR. J Clin Microbiol 1999; 37: 1469-73.
11. Socransky SS, Smith C, Haffajee AD. Subgingival microbial profiles in refractory periodontal disease. J Clin Periodontol 2002; 29: 260-8.
12. Hodge PJ, Riggio MP, Kinane DF. Failure to detect an association with IL-1 genotypes in European Caucasians with generalised early onset periodontitis. J Clin Periodontol 2001; 28: 430-6.
13. Papapanou PN, Neiderud AM, Sandros J, Dahlen G. Interleukin-1 gene polymorphism and periodontal status. A case-control study. J Clin Periodontol 2001; 28: 389-96.
14. Greenstein G, Hart TC. A critical assessment of interleukin-1 (IL-1) genotyping when used in a genetic susceptibility test for severe chronic periodontitis. J Periodontol 2002; 73: 231-47.
15. Shapira L, Wilensky A, Kinane DF. Effect of genetic variability on the inflammatory response to periodontal infection. J Clin Periodontol 2005; 32 Suppl: 72-86.
16. Konig J, Ruhling A, Plagmann HC, Meisel P, Kocher T. Influence of interleukin (IL)-1 composite genotype on clinical variables in non-smoking, well-maintained compliant patients with chronic periodontitis. Swed Dent J 2005; 29: 11-6.
17. Cullinan MP, Westerman B, Hamlet SM, Palmer JE, Faddy MJ, Lang NP, Seymour GJ. A longitudinal study of interleukin-1 gene polymorphisms and periodontal disease in a general adult population. J Clin Periodontol 2001; 28: 1137-44.
18. Thomson WM, Edwards SJ, Dobson-Le DP, Tompkins GR, Poulton R, Knight DA, Braithwaite AW. IL-1 genotype and adult periodontitis among young New Zealanders. J Dent Res 2001; 80: 1700-3.
19. Socransky SS, Haffajee AD, Smith C, Duff GW. Microbiological parameters associated with IL-1 gene polymorphisms in periodontitis patients. J Clin Periodontol 2000; 27: 810-8.
20. Kawada M, Yoshida A, Suzuki N, Nakano Y, Saito T, Oho T, Koga T. Prevalence of Porphyromonas gingivalis in relation to periodontal status assessed by real-time PCR. Oral Microbiol Immunol 2004; 19: 289-92.
21. Klein MI, Goncalves RB. Detection of Tannerella forsythensis (Bacteroides forsythus) and porphyromonas gingivalis by polymerase chain reaction in subjects with different periodontal status. J Periodontol 2003; 74: 798-802.