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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