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ORIGINAL ARTICLE

IL-1-polymorphism and severity of periodontal disease

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Abstract

Objective. To determine the association between the interleukin (IL)-1-polymorphism and the severity of periodontal disease prior to active periodontal therapy. ***Materials and methods.*** Two hundred and six patients with obtained baseline x-rays were tested for IL-1-polymorphism. Relative bone loss before active periodontal treatment was measured with a Schei ruler and classified in five groups. Descriptive statistics and backward stepwise linear regression analyses were performed. ***Results.*** Forty-nine patients with moderate (mChP), 79 with severe chronic (sChP) and 78 with aggressive periodontitis (AgP) were included. Age correlated significantly with bone loss and number of teeth at baseline. Gender, smoking and IL-1-polymorphism were neither associated with bone loss nor with number of teeth prior to treatment. After adjusting for age as well as gender, AgP was significantly associated with more severe bone loss in untreated periodontal disease ($p = 0.036$). In non-smokers, mean number of teeth prior to active periodontal therapy correlated significantly with presence of IL-1 polymorphism. ***Conclusion.*** The IL-1-polymorphism is associated with lower number of teeth in non-smokers with untreated periodontal disease. Untreated AgP is associated with more severe bone loss than untreated ChP.

Key Words: *interleukin-1, baseline diagnosis, smoking, severity, periodontitis*

Introduction

It has been demonstrated by epidemiological studies that the severity of periodontitis differs considerably among subjects of the same population [1,2]. Several parameters contribute to the inter-individual differences in this multi-factorial disease: cigarette smoking presents a well-established environmental risk factor and psychosocial stress poses as a putative risk factor [3,4]. Additionally, local (bacterial biofilms) as well as systemic/genetic components (e.g. diabetes mellitus, cytokine gene polymorphisms) play an important role in the progression of the disease [5,6]. Whereas the influence of diabetes mellitus and smoking is well established, the significance of the interleukin-1 (IL-1) polymorphism regarding the severity of periodontal diseases remains controversial [7]: an association between a more pronounced periodontal decline in non-smokers and the IL-1 polymorphism (termed 'composite genotype') was first documented by Kornman et al. [8]. Several studies affirm this observation [9,10], whereas other authors report on controversial results of a gene-environmental interaction between smoking and IL-1-polymorphism with an increased risk for periodontitis [11,12] or did not document any association [13].

Recent studies reporting on the influence of the IL-1-polymorphism in patients with aggressive periodontitis (AgP) present more defined results: a cohort of 415 Caucasians suffering from AgP and 874 periodontally healthy controls were compared regarding polymorphisms in the genes encoding for interleukin-1α (-889) and interleukin-1β ($+3953$). No association between variants in the IL-1 gene cluster and AgP could be found [14]. Similar results were documented by Scapoli et al. [15]. In summary,

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an association between AgP and IL-1 polymorphism seems non-existent, whereas the influence in chronic periodontitis (ChP) still remains contradictory.

The aim of the present study was to determine the influence of the IL-1 polymorphism and other factors (e.g. smoking, gender) on the severity of periodontal disease with regard to baseline diagnosis.

Materials and methods

Patients

In this bi-centric study, files of 206 patients, 134 women and 72 men, with aggressive or chronic periodontitis were searched. All patients had received active periodontal treatment (anti-infective therapy with sub-gingival debridement under local anesthesia and if required periodontal surgery) at the Section of Periodontology at the Department of Conservative Dentistry, Clinic for Oral, Dental and Maxillofacial Disease at the University Hospital Heidelberg or at the Department of Periodontology of the Center of Dental, Oral and Maxillofacial Medicine (Carolinum), Johann Wolfgang Goethe-University Frankfurt/Main between 1992–2005. All patients fulfilled the following inclusion criteria:

- Existence of baseline x-rays (panoramic radiograph or x-ray status),
- Availability of IL-1-Genotype test result,
- Patients older than 18 years, and
- Written informed consent.

Additionally, patients with aggressive periodontitis had to fulfil further criteria:

- Baseline x-rays showed inter-proximal bone loss of ≥ 50% at two or more teeth,
- A non-contributory medical history at baseline, and
- ≤ 35 years at baseline.

The study was conducted according to the Declaration of Helsinki (1964, revision 2008) and approved by the Institutional Review Board for Human Studies of the Medical Faculty of Heidelberg University (Application# S-134/2009).

Evaluation of patients' charts

Retrospectively, patient-related data (gender, age, periodontal diagnosis, smoking habits, IL-1-Genotype, number of teeth) as well as tooth-related data (type of tooth, vertical clinical attachment level [PAL-V] at six sites per tooth) were recorded by one independent examiner (NES).

Periodontal baseline diagnosis (moderate/severe chronic periodontitis or aggressive periodontitis) was in part retrospectively assigned to each individual according to the actual classification of periodontal diseases [16]. If information on PAL-V was missing in the chart, it was replaced by interproximal bone loss detected in baseline radiographs [17]. According to their smoking habits, patients were classified as smoker or non-smoker. Non-smokers included patients who had never smoked or quit smoking at least 5 years before therapy was initiated. Individuals who had quit smoking less than 5 years ago were classified as smokers.

Analysis of genetic polymorphism

All patients were tested for interleukin-1-polymorphism (IL-1A −889, IL-1B +3953) using one of two different test kits (GenoType PRT Parodontitis-Risiko-Test, Hain Life Science GmbH, Nehren, Germany or IAI ParoGen Test, IAI Institut für Angewandte Immunologie, Zuchwil, Switzerland). A patient was classified as IL-1 positive if both the second allele for IL-1A and IL-1B was positive. The excellent agreement ($\kappa = 1.0$) of both laboratories had been confirmed with a double-testing in 10 patients [17].

For DNA-isolation a sterile foam swab was rubbed over cheek mucosa for 30 s to sample cells and sent to the laboratory where analysis was carried out with polymerase chain reaction. Patients who showed at least one copy of the variant allele 2 at position IL-1A −C889T (rs1800587) and IL-1B +C3953T (rs1143634) were classified as IL-1-polymorphism positive (i.e. 'composite genotype' as defined by Kornman et al. [8]).

Evaluation of radiographs

Prior to active periodontal treatment, complete sets of periapical radiographs (Ultraspeed; Kodak, Rochester, NY) were obtained by XCP technique using film holders (XPC, Kentzler & Kaschner Dental, Ellwangen/Jagst, Germany). Dental films were exposed to an x-ray source (Heliodent 70®, 70 kV, 7 mA, Sirona, Bensheim, Germany) and developed under standardized conditions (Periomat®, Dürr Dental, Bietigheim-Bissingen, Germany). Alternatively, if an intra-oral x-ray status was not obtained, the baseline panoramic radiograph (Orthophos Plus® or Orthophos DS Ceph®, 60–90 kV, 9–16 mA, Siemens, Germany) was used for evaluation of bone loss.

All radiographs were examined in a darkened room using a radiograph screen (67-0420, Dentsply Rinn, Elgin, IL) or in the case of digital x-rays using a standardized personal computer (Friacom-PC, Friadent AG, Mannheim, Germany). At each tooth, relative interproximal bone loss was measured at the most affected site in relation to the root length using a Schei ruler [18]. Toehold of the Schei ruler was the cemento-enamel-junction (CEJ). If that was covered

by a restoration the restoration margin (RM) was taken as landmark. According to their periodontal bone loss (CEJ/RM to bony defect) teeth were classified in one of five groups according to their periodontal bone loss [19]:

- Group 1: < 20%,
- Group 2: 20% to < 40%,
- Group 3: 40% to < 60%,
- Group 4: 60% to < 80%, and
- Group 5: 80% and more.

All radiographic assessments were conducted by two independent examiners blinded regarding baseline diagnosis (RC, JK).

Statistical analysis

All data was entered by two investigators (NES, RC) into two separate data files (Excel version 2003, Microsoft Corporation, Redmond, WA). Both data files were compared by subtraction of columns of identical variables. If subtraction resulted in values different from null the entries were double-checked by means of comparison with the original patient's charts and corrected.

The patient was looked upon as a statistical unit and bone loss at baseline was defined as the main outcome variable. The second outcome variable was number of teeth prior to active periodontal therapy.

Keeping in mind that periodontal status is strongly related to smoking status the sample was divided into four groups: (1) IL-1 positive non-smokers, (2) IL-1 negative non-smokers, (3) IL-1 positive smokers and (4) IL-1 negative smokers. For the total sample and the four sub-groups separately distribution of genders and diagnoses (mChP, sChP, AgP) were described in total numbers as well as percentage and compared using χ^2 tests. Means were calculated for age, number of teeth and bone loss at baseline. These means were compared using ANOVA with post-hoc tests using Bonferroni adjustment. Due to the unbalanced distribution according to age between the different groups, backward stepwise linear regression analyses were performed for the dependent variables mean number of teeth and mean bone loss at baseline using the independent variables gender, age, diagnosis (ChP/AgP) and group (IL-1 positive non-smokers/IL-1 negative non-smokers/IL-1 positive smokers/IL-1 negative smokers). A type 1 error α of 0.1 was accepted to keep a variable in the model. Statistical analysis was performed using a computer program (SPSS, Version 18, SPSS Inc., Chicago IL). Third molars were excluded from analysis.

Results

One hundred and thirty-four female and 72 male patients who were tested for IL-1 polymorphism and for whom x-rays had been obtained prior to active periodontal therapy at the Section of Periodontology, University Hospital Heidelberg or at the Department of Periodontology, Johann Wolfgang Goethe-University Frankfurt/Main were included in this retrospective study. The diagnosis moderate chronic periodontitis (mChP) was assigned to 49 individuals (23.8%), 79 patients (38.3%) suffered from severe chronic periodontitis (sChP) and 78 participants (37.9%) were classified as suffering from aggressive periodontitis. Fifty-four individuals were smokers and in 79 patients (38.3%) a positive IL-1 polymorphism was expressed, which slightly exceeds the prevalence of 33% in Central Europe as reported by De Sanctis and Zucchelli [20] (Table I).

Table I. Patient's characteristics according to smoking habits and IL-polymorphism.

		Non-smokers, 152 (74)		Smokers, 54 (26)		
	Total	IL-1 positive	IL-1 negative	IL-1 positive	IL-1 negative	*p*
n (%)	206	54 (26)	98 (48)	25 (12)	29 (14)	
Gender						n.s
Female, *n* (%)	134 (65)	29 (54)	63 (64)	20 (80)	22 (76)	
Male, *n* (%)	72 (35)	25 (46)	35 (36)	5 (20)	7 (23)	
Age (years)	41.48 ± 11.8	43.91 ± 13.2	43.12 ± 12.0	34.08 ± 7.7*	37.76 ± 7.9	0.001
Diagnosis						0.039
Moderate ChP, *n* (%)	49 (24)	14 (26)	28 (29)	2 (8)	5 (17)	
Severe ChP, *n* (%)	79 (38)	26 (48)	36 (37)	8 (32)	9 (31)	
AgP, *n* (%)	78 (38)	14 (26)	34 (34)	15 (60)	15 (52)	0.010
Mean bone loss at baseline	2.12 ± 0.541	2.04 ± 0.48	2.09 ± 0.47	2.29 ± 0.83	2.22 ± 0.54	n.s
Mean number of teeth at baseline	24.4 ± 4.1	23.2 ± 4.5	24.6 ± 3.1	25.8 ± 4.7	24.5 ± 5.2	n.s

ChP, chronic periodontitis; AgP, aggressive periodontitis.
*Statistically significantly different from IL-1 positive and negative non-smokers.

Analysis failed to identify an unbalanced distribution of genders between the four groups. IL-1 positive smokers were statistically significantly younger than IL-1 positive and negative non-smokers. Diagnoses (mChP, sChP, AgP) were statistically significantly unequally distributed between groups with a higher amount of sChP and AgP in IL-1 positive smokers than in IL-1 negative and positive non-smokers. Unadjusted comparisons of the four groups failed to identify statistically significant differences regarding mean bone loss and mean number of teeth at baseline (Table I).

Backward multiple linear regression analyses particularly adjusting for age as well as gender and diagnosis (ChP/AgP) identified AgP ($p = 0.036$) to be statistically significantly associated with more severe bone loss in untreated periodontal disease (Table II). Mean number of teeth prior to active periodontal therapy was statistically significantly associated with age ($p < 0.001$) and IL-1 positive non-smokers ($p = 0.058$) (Table III). On average, IL-1 positive non-smokers showed 23.2 ± 4.5 teeth before treatment, whereas IL-1 negative non-smokers displayed 24.6 ± 3.1 teeth.

Discussion

The importance of genetic factors regarding the individual variability of periodontitis has been demonstrated by studies in twins [21]. The influence of the IL-1 polymorphism as one genetic risk factor remains controversial. Whereas some studies document an association between the severity of periodontal disease and the IL-1 polymorphism [8,9,22] other studies reject it [14,23–25]. A recent review by Nikolopoulos et al. [26] found a moderate statistically significant association between chronic periodontitis and IL-1A -889T and IL-1B 3953/4T in Caucasians.

It has been discussed previously that the effect of the IL-1 polymorphism may be obscured by stronger risk factors like smoking or infection with periodontal pathogens [10]. Other studies indicated that the IL-1 polymorphism only has an impact on the severity of periodontal disease in conjunction to other risk factors, i.e. smoking [12]. Thus, we sub-divided our sample into four groups according to IL-1 polymorphism (yes/no) and smoking (yes/no). Performing comparisons in our study sample of 206 individuals without adjustment for confounders like age and presence/absence of the IL-1 polymorphism did not result in statistically significant differences with regard to bone loss or number of teeth in untreated periodontitis patients. However, IL-1 positive smokers were significantly younger than IL-1 positive and negative non-smokers. Age is a well-established background factor of periodontal disease status [17,27]. Two factors affecting periodontal status were cancelling each other out in the smoker group, i.e. young age being associated with less periodontal disease and smoking being associated with increased periodontal disease. This imbalance regarding age may have obscured any difference between the groups with regard to bone loss and number of teeth.

Backward linear multiple regression analysis identified AgP as a risk indicator for mean bone loss in untreated patients. Analysis failed to identify IL-1 polymorphism as a risk indicator for mean bone loss. However, IL-1 polymorphism in smokers and age could be detected as risk indicators for tooth loss (i.e. reduced number of teeth) in untreated patients. These results corroborate those by Kornman et al. [8], who tested 134 Caucasians of Northern European heritage for the so-called 'composite genotype' and documented an association in non-smokers with severe periodontal disease [8]. These findings were supported by McDevitt et al. [9], who showed an odds ratio of 3.75 for moderate-to-severe periodontitis in IL-1 genotype positive non-smokers compared with IL-1 genotype negative smokers. Indeed, in a further study by Papapanou et al. [13] the IL-1 polymorphism failed to distinguish between periodontally diseased patients and controls, but an association between the severity of periodontal disease and the presence of the IL-1 polymorphism could be demonstrated in never smoking patients. Contrary results were presented by Meisel et al. [12] in a huge cohort of 1085 individuals: IL-1 genotype positive smokers were detected to be at an increased risk for periodontitis, whereas in non-smokers the IL-1 polymorphism showed no association to severity of the disease.

Smoking acts as one of the major risk factors for periodontal disease [3,28–30]. In conclusion with the

Table II. Mean bone loss at baseline in relation to age, gender, diagnosis, interleukin-1 polymorphism and smoking.

	b	SE (*b*)	*T*	*p*
Constant	2.070	0.054	38.066	< 0.001
Aggressive periodontitis	0.186	0.088	2.106	0.036
Analysis of variance: $p < 0.036$				

Dependent variable: Mean bone loss at baseline; $n = 206$; $R^2 = 0.021$; $R^2_{adjusted} = 0.016$; standard error of estimate = 0.615.

Table III. Mean number of teeth at baseline in relation to age, gender, diagnosis, interleukin-1 polymorphism and smoking.

	b	SE (*b*)	*T*	*p*
Constant	30.363	0.946	32.080	< 0.001
Age	−0.137	0.022	−6.198	< 0.001
Interleukin-1 positive and non-smoking	−1.129	0.593	−1.904	0.058
Analysis of variance: $p < 0.001$				

Dependent variable: Mean number of teeth at baseline; $n = 206$; $R^2 = 0.183$; $R^2_{adjusted} = 0.175$; standard error of estimate = 3.716.

aforementioned studies in our sample, cigarette smoking may have obscured the effect of the genetic predisposition to an exaggerated host response by IL-1 polymorphism. Thus, the presence of the IL-1 polymorphism acts as an indicator for tooth loss, particularly in non-smoking patients [7].

In accordance with our results, an association between AgP and IL-1-polymorphism could not be established in two recent studies either. Fiebig et al. [14] examined 415 Caucasian patients with AgP and 874 controls regarding polymorphisms in the IL-1 gene cluster. No association between variants in the IL-1 gene cluster and AgP could be found and it was suggested to interpret previous reports showing opposite results with care. This was corroborated by Scapoli et al. [15] who could not demonstrate any associations in AgP, either. Gore et al. [31] documented that the IL-1 polymorphism affects the severity of periodontal disease in adult periodontitis. This coincides with our results, because IL-1-polymorphism as a significant risk indicator for bone or tooth loss was just found in patients with mChP.

However, it has to be kept in mind that our sample of 206 patients as well as study samples in most of the mentioned studies may be too small to correctly prove the impact of genetic risk factors [32]. As concluded by Laine et al. [10], other risk indicators may have obscured the influence of a positive IL-1 polymorphism.

In conclusion, our findings confirm the influence of the IL-1 polymorphism on the number of teeth in non-smokers with untreated periodontitis. Also, the IL-1 polymorphism was not in any sub-group or the whole study sample associated with bone loss, whereas AgP could be confirmed as a statistically significant risk indicator for bone loss.

Clinical relevance: The IL-1-polymorphism depicts a risk indicator for tooth loss in non-smoking patients with untreated periodontal disease.

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