Interleukin-1 gene polymorphisms and periodontal status in a Spanish population

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Abstract. The aim of this study was to investigate the possible association between interleukin (IL)-1A (+4845) and/or IL-1B (+3954) gene polymorphisms and the onset and progression of chronic periodontal disease (PD), an issue that remains controversial. The relationship between IL-1β concentration in the gingival crevicular fluid (GCF) and disease activity was also evaluated. The study was performed on 25 individuals with no gingivitis or PD and on 25 subjects with active chronic PD. Two samples of GCF were obtained from each subject and IL-1β was determined by enzyme-linked immunoabsorbent assay. Blood samples (10 ml) were drawn from each subject to detect polymorphisms in IL-1A (+4845) and IL-1B (+3954) by polymerase chain reaction. Mean GCF IL-1β concentrations were higher in patients with active chronic PD compared to the control group. No significant association was found in either group between GCF IL-1β concentration and the presence of polymorphisms in IL-1A (+4845), IL-1B (+3954) or both genotypes. No significant difference was found in either group with regard to the presence of polymorphisms in IL-1A (+4845), IL-1B (+3954) or both genotypes (p=0.556). The concentration of IL-1β in GCF was almost 2-fold higher in patients with chronic PD than in the healthy individuals. The presence of polymorphisms in IL-1A (+4845) and/or IL-1B (+3954) genotypes is not associated with IL-1β overproduction in GCF and is not a risk factor for chronic PD. IL-1β is considered a suitable marker of the severity and progression of chronic PD. The presence of IL-1A (+4845) and/or IL-1B +3954 gene polymorphisms does not appear to be a risk factor for chronic PD. Therefore, the IL-1A (+4845) and/or IL-1B +3954 gene polymorphisms cannot be considered genetic markers of chronic PD. Moreover, these polymorphisms do not indicate an overproduction of IL-1β in GCF.

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Introduction

Periodontal disease (PD) is a chronic inflammatory disease generated by a series of specific periodontopathogenic bacteria (1-4). Interleukin-1 (IL-1), together with other factors, is involved in the onset of tooth insertion tissue destruction (5,6), and its synthesis is closely associated with PD severity and progression. Several studies have demonstrated that individuals exposed to the same environmental risk factors and with similar levels of dental care differ in their predisposition to PD (7,8). Numerous cell families secrete IL-1, whose production is genetically determined by *IL-1A*, *IL-1B* and *IL-1RN* (9). Various polymorphisms in these genes have been associated with changes in the production of the corresponding proteins, IL-1α, IL-1β and IL-1ra (10,11).

IL-1β inhibits bone formation by stimulating the synthesis of prostaglandins and tromboxans and the production of collagen and proteases (12,13). *In vitro* studies have shown that IL-1β is 15-fold more potent than IL-1α, and 500-fold more potent than TNF-α in the inhibition of bone resorption (14). Moreover, 10- to 50-fold greater amounts of IL-1β than IL-1α were isolated in periodontitis gingival crevicular fluid (GCF) from sites with PD (15).

An association between IL-1 polymorphisms and PD was reported by certain authors (10,16,17). However, other authors found no such correlation (18-20), thus the issue remains controversial. The aim of the present study was to examine the association between IL-1β in GCF and PD activity and to investigate the presence of IL-1 gene polymorphisms and their possible relationship with the disease, in a Spanish population.

Patients and methods

Patients. This observational, cross-sectional case-control study was carried out on students of a Periodontics Master's course at our School of Dentistry. Two gender-matched groups of 25 patients each (12 males, 13 females) were randomly selected among the students with no gingivitis or periodontitis and those with moderate/severe PD, respectively. General inclusion criteria for the two groups were: subjects and their parents had to be of Spanish origin, subjects had to be non-smokers or ex-smokers for >5 years, subjects had to

be free of any systemic disease (e.g., diabetes, HIV, immunological or haemorrhagic disorders), subjects were required not to have taken any antibiotics in the past 4 months, as well as be free of any chronic medication and not be pregnant or breastfeeding. Specific inclusion criteria for the healthy group were: absence of gingivitis, insertion loss ≤3 mm and absence of periodontal pockets.

Inclusion criteria for the PD group were: loss of insertion ≥5 mm in ≥2 teeth in each quadrant, pocket depth ≥6 mm in ≥1 tooth per quadrant, radiographic evidence of bone loss of ≥30%, presence of ≥20 teeth in the mouth (excluding third molars) with ≥1 molar in each quadrant, and no periodontal treatment in the past year. The age range was 25-51 years in the PD group and 25-47 years in the healthy group.

Sample collection. Two samples were collected from the participants; from the mesiobuccal aspects of the two first upper molars in the healthy group, and from the deepest probing site showing disease activity, as defined by Lang *et al* (21), in each upper quadrant, in the PD group. To obtain the samples, the area was isolated with cotton rolls and air-dried with a syringe for 5 sec at 45˚ to the tooth root without desiccating the gingival groove. Subsequently, a paper strip (Harco Electronics, Irving, CA, USA) was introduced into the groove and left in place for 30 sec, avoiding contamination with blood or sputum. The strip was analyzed in a Periotron 8000 machine (Harco Electronics). Samples were stored at -70˚C. IL-1β levels were measured using a streptavidin-peroxidase ELISA kit (Biosource Inc., Camarillo, CA, USA), according to the manufacturer's instructions. Immunodetection was performed by mixing 50 μl of sample with 100 μl of anti-IL-1β solution and quantifying the signal spectrophotometrically at a wavelength of 450 nm.

Blood samples. A 10-ml sample of venous blood was obtained from all patients and submitted to standard analysis, verifying the absence of systemic diseases. An aliquot of this sample was used to isolate DNA as described by Miller *et al* (22).

Genetic polymorphism analysis. The presence of polymorphisms in the IL-1A and IL-1B genes was tested by the PCR amplification of DNA fragments encompassing the polymorphic site, followed by restriction and separation of the DNA fragments using electrophoresis on agarose gels (23). The specific conditions were as follows:

IL-1A (+4845). This polymorphism involves the substitution of G by T at the 4845 position, introducing a *Fnu*4H1 recognition site. The primers used for amplification were: forward: 5'-ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA and reverse: 5'-AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT (23). The reaction was carried out in 50 μl using *Taq* polymerase Gibco® 5 IU, 25 μM $MgCl_2$, 10 μM dNTPs, 10 μM of each primer and the reaction buffer (1X) supplied with the kit. Reactions were performed on a DNA thermal cycler (PCR system Perkin Etmer 2400) with the following thermal profile: 1 cycle of denaturation at 95˚C for 10 min, 35 cycles at 94˚C for 30 sec, 53˚C for 30 sec, 74˚C for 30 sec and an extension step at 72˚C for 3 min. The 153-bp amplified DNA was digested with the restriction endonuclease *Fnu*4H1, and the resulting products were visualized by electrophoresis on 1.5 and 3% agarose gels stained with ethidium bromide. The polymorphism was detected by the appearance of two fragments of 124 and 29 bp.

Table I. Clinical characteristics of periodontal patients and controls.

	Mean	Mean standard error	Median	Standard deviation
Case group (n=25)				
IL-1β left (pg/μl)	4.16	0.57	2.99	2.85
IL-1β right (pg/μl)	4.02	0.56	2.83	2.81
Probing depth left	6.20	0.16	6.00	0.82
Probing depth right	6.24	0.23	6.00	1.13
Bleeding index	74.69	3.00	74.80	15.02
Plaque index	77.07	3.78	78.10	18.92
Age (years)	41.96	1.66	43.00	8.31
Control group				
IL-1β left (pg/μl)	2.30	0.29	1.84	1.46
IL-1β right (pg/μl)	2.38	0.28	1.93	1.42
Probing depth left	3.00	0.00	3.00	0.00
Probing depth right	3.00	0.00	3.00	0.00
Bleeding index	8.17	0.76	8.10	3.79
Plaque index	7.76	0.68	7.30	3.38
Age (years)	36.56	1.35	36.00	6.75

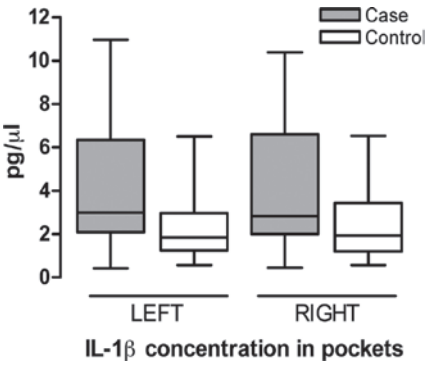

Figure 1. Distribution of IL-1β concentrations in the left and right pockets. Columns show the median value as a horizontal line. Error bars show the upper and lower limits of the 95% confidence interval.

IL-1B (+3954). This polymorphism is characterized by the substitution of C by T at the +3953 position, leading to the appearance of a *Taq*1 site. The primers used were: forward: 5'-CTC AGG TGT CCT CGA AGA AAT CAA and reverse: 5'-GCT TTT TTG CTG TGA GTC CCG, producing an amplicon of 182 bp. The amplified reaction mixture (50 μl) contained 10X PCR buffer, 25 μM $MgCl_2$, 10 μM dNTPs, 10 μM of each primer and 5 IU of PCR *Taq* polymerase (Gibco). The reactions were performed as above, but using 50˚C as the annealing temperature. The amplified DNA was digested with restriction endonuclease *Taq*1 and the resulting products were visualized on agarose gels, as above. Expected fragment sizes in homozygotes carrying the polymorphism were 97 and 85 bp.

Table II. Concentrations of GCF IL-1β in the left and right pockets in the PD and control groups.

Group	No.	Mean (pg/μl)	Bilateral asymptotic significance (p)
IL-1β left			
PD	25	30.48	0.016
Control	25	20.52	
IL-1β right			
PD	25	29.76	0.039
Control	25	21.24	

Table III. Distribution of IL-1A (+4845) and IL-1B (+3954) polymorphisms in the study population.

	IL-1A [no. (%)]	IL-1B [no. (%)]
1.1	27 (54)	28 (56)
1.2	18 (36)	20 (40)
2.2	5 (10)	2 (4)
Total	50 (100)	50 (100)

1.1, non-polymorphic homozygote; 1.2, heterozygote; 2.2, polymorphic homozygote.

Table IV. Prevalence of polymorphisms in the two genes in the study population.

Genotype	No.	%
Negative	32	64
Positive	18	36
Total	50	100

Positive, at least one polymorphic allele at IL-1A and one polymorphic allele at IL-1B; negative, at least one non-polymorphic gene.

Statistical analysis. Since the Kolmogorov-Smirnov test showed an abnormal distribution, the data were analyzed with non-parametric tests: the Spearman Rho correlation, Kruskal-Wallis, Mann-Whitney U and Chi-square tests. P<0.05 was considered statistically significant. SPSS 12.0 (SPSS Inc., Chicago, IL, USA) was used for the data analyses.

Results

Table I summarises the clinical characteristics of the PD and the control group. Mean probing depths, bleeding and plaque indices were consistently higher in the PD than in the control group. GCF IL-1β concentrations were almost 2-fold higher in the PD group than in the control group (left pocket: 4.161±2.854 vs. 2.3±1.5 pg/μl, p=0.039; right pocket: 4.0±2.8 vs. 2.4±1.4 pg/μl, p=0.016) (Fig. 1, Table II).

In the global samples (cases and controls), 46% were shown to be IL-1A (+4845) gene polymorphisms, 44% IL-1B (+3954) polymorphisms and 36% both polymorphisms (Tables III and IV). As shown in Table V, the groups did not significantly differ in the prevalence of IL-1A (+4845) or IL-1B (+3954) polymorphisms (p=0.504) or both (positive genotype).

No significant correlations were found between GCF IL-1β concentrations and probing depth, insertion loss, plaque index or bleeding index, between GCF IL-1β concentrations

Table V. Distribution of IL-1A (+4845) and IL-1B (+3954) polymorphisms between patients with PD and healthy controls.

	IL-1A			IL-1B			Genotype[a]	
	1.1	1.2	2.2	1.1	1.2	2.2	No	Yes
PD (%)	64	28	8	64	32	4	68	32
Control (%)	44	44	12	48	48	4	60	40

[a]Comparison between groups for IL-1A (+4845) polymorphism (χ^2=2.015, p=0.365), IL-1B (+3953) polymorphism (χ^2=1.371, p=0.504) and positive/negative genotype (χ^2=0.347, p=0.556).

Table VI. Mean range of IL-1β concentrations (pg/μl) in the GCF of subjects with IL-1A (+4845) and/or IL-1B (+3954) alleles.

	Heterozygosis	IL-1A	IL-1B
IL-1β left	1.1	25.74	25.89
	1.2	24.61	25.20
	2.2	27.40	23.00
IL-1β right			
	1.1	25.96	25.89
	1.2	24.56	25.00
	2.2	26.40	25.00

IL-1A (+4845): left pocket, χ^2=0.159, p=0.923; right pocket, χ^2=0.122, p=0.941; IL-1B (+3953): left pocket, χ^2=0.088, p=0.957; right pocket, χ^2=0.046, p=0.977.

Table VII. Mean range of IL-1β concentrations (pg/μl) in the GCF of subjects with positive or negative genotypes.

	Genotype	Mean
IL-1β left	Positive	25.53
	Negative	25.44
IL-1β right	Positive	25.91
	Negative	24.78

Left, Mann-Whitney U test = 287.000, p=0.984; right, Mann-Whitney U test = 275.000, p=0.793.

and the IL-1A (+4845) polymorphism, between GCF IL-1β and the IL-1B (+3954) polymorphism, or between GCF IL-1β concentrations and polymorphisms in the two genes (Tables VI and VII).

Discussion

Relationship between GCF IL-1β and periodontal status. In this study, IL-1β was detected in the GCF of the studied sites of the subjects, as has also been reported by Preiss and Meyle (24), and Rawlison *et al* (25). By contrast, Suwatanapongched *et al* (26) and Wilton *et al* (27) failed to detect IL-1β in GCF at healthy sites. This discrepancy may result from the use of different sampling methods and/or ELISA kits. GCF IL-1β concentrations were almost 2-fold higher ($p<0.05$) in our PD patients than in the healthy controls, consistent with reports of a strong relationship between IL-1β and periodontal inflammation (26,28-31). Masada *et al* (32), in a study of 15 patients with moderate-to-severe PD, found that their elevated GCF IL-1α and IL-1β concentrations were reduced with periodontal treatment. Salvi *et al* (33) reported higher GCF IL-1β concentrations in patients with moderate-to-severe periodontitis than in patients with gingivitis and moderate periodontitis.

Giannopoulou *et al* (34) also observed higher concentrations of GCF IL-1β in PD patients than in gingivitis patients and healthy controls, and reported higher levels in active vs. non-active sites among the PD patients; healthy sites showed higher levels of IL-4, which is considered to have a protective effect against PD, whereas IL-1β, IL-6 and IL-8 were associated with periodontal destruction.

Relationship between IL-1 polymorphisms and periodontal status. No significant difference was found between the groups with regard to the presence of IL-1 gene polymorphisms. This result is in line with the report by Rogers *et al* (18) whose findings indicated no difference among groups with chronic PD, early-onset periodontitis, and no PD in the presence of IL-1A (+4845) polymorphism or positive genotype. These authors did not detect any correlation between IL-1 polymorphisms and susceptibility to implant loss. Results of a study by Papapanou *et al* (19) found no differences in the prevalence of positive or negative genotypes between PD patients and healthy individuals. By contrast, Galbraith *et al* (16) and Gore *et al* (10) observed a correlation between the IL-1B (+3953) polymorphism and the presence of advanced PD when smokers were included in the study group, although the relationship did not reach statistical significance, stating that individuals with this polymorphism are 3-fold more susceptible to PD. Kornman *et al* (17) found a significantly higher prevalence of positive genotypes in non-smokers with severe PD, and Shirodaria *et al* (35) reported a significant association between the IL-1A polymorphism (+4845) and a higher risk of severe PD in non-smokers. However, findings of studies from 3 consecutive years (20,36,37) showed that only smokers with positive IL-1 genotype were at a higher risk of PD, concluding that tobacco use is a more important risk factor than genetics. Drozdzik *et al* (38) found no significant association between the presence of the IL-1B polymorphism (+3953) and the periodontal status of patients, consistent with the present study, describing plaque index and age as more influential risk factors. By contrast, Agrawal *et al* (39) reported that the presence of IL-1 gene polymorphisms is a clear risk factor for chronic PD, finding significant differences between healthy non-smokers and non-smoking patients with severe chronic PD. A recent review by Huynh-Ba *et al* (40) reflected the discrepancies among published clinical findings and concluded that the evidence on the contribution of IL-1 gene polymorphisms on PD progression was inadequate. These authors also suggested that the results of certain commercial genetic tests should be interpreted with caution.

Correlation between the GCF IL-1β concentration and the presence of polymorphisms. No significant correlation between GCF IL-1β and any polymorphism studied was found. This observation is consistent with previous reports whereby positive or negative IL-1 genotypes have no effect on IL-1β production in monocytes exposed to periodontal pathogens (41). However, reports on this relationship have been controversial, with findings of a decrease (42) and an increase (10) in IL-1β production by peripheral mononuclear cells in individuals with the IL-1β (+3953) polymorphism. Pociot *et al* (11) found that, following exposure to *Escherichia coli* lipopolysaccharides, 30-40% more IL-1β was produced in IL-1B (+3953) heterozygotes and 50% more in IL-1B (+3953) homozygotes compared to those with a negative genotype, regardless of their periodontal status.

In conclusion, GCF IL-1β concentrations are higher in individuals with active chronic PD than in healthy individuals. GCF IL-1β concentrations do not differ among PD-affected pockets in the same individual. IL-1β is considered a suitable marker of the severity and progression of chronic PD. The presence of IL-1A (+4845) and/or IL-1B +3954 gene polymorphisms does not appear to be a risk factor for chronic PD. Moreover, IL-1A (+4845) and/or IL-1B +3954 gene polymorphisms cannot be considered genetic markers of this condition and they do not indicate an overproduction of IL-1β in GCF. Nevertheless, further study on genetic variations in different populations is required to elucidate the role of genetic factors in the onset and progression of periodontal disease.

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