Original Article

Association of IL-8 (-251 A/T) Gene Polymorphism with Clinical Parameters and Chronic Periodontitis

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Abstract

Objective: To investigate the correlation between IL-8 (-251 A/T) gene polymorphism and susceptibility to chronic periodontitis as well as different clinical parameters and severity of the condition in patients referred to dental school, Shiraz University of Medical Sciences, Shiraz, Iran.

Materials and Methods: In this randomized cross sectional study, 227 non-smoking patients with chronic periodontitis (test) and 40 healthy individuals (control) were enrolled in this experiment and the following clinical parameters were employed in the study: Periodontal Pocket Depth (PPD), Clinical Attachment Level (CAL) and Bone Loss (BL). All participants underwent the PCR (Polymerase Chain Reaction) test to detect 251 A/T Single Nucleotide Polymorphism of IL8 gene.

Results: No significant correlation was perceived between different genotypes of IL-8 and the severity of the periodontal condition (P= 0.164), neither did we detect any substantial association between different IL-8 genotypes and the mean PPD (P=0.525), CAL (P=0.151), BL (P=0.255), PI (P=0.087), BOP (P=0.265) and the average number of teeth (P=0.931).

Conclusion: The results implied that there was no explicit correlation between 251 (A/T) IL-8 gene polymorphism and the severity of the chronic periodontal disease or to the susceptibility to it.

Key Words: Genetic polymorphism; Interleukin-8; Chronic Periodontitis

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INTRODUCTION

Cytokines play an imperative role in tissue deterioration in periodontal diseases. Recent investigations have confirmed a correlation between genetic markers, incidence and severity of periodontal diseases. Genetic polymorphism in cytokine genes is regarded as a promising factor in inducing periodontal diseases [1].

IL-8 is a pre-inflammatory cytokine related to the initiation and intensification of acute and chronic inflammatory process. Its responsibility in the pathology of periodontal diseases has been formerly investigated.

It is typically released by numerous cells such as lymphocytes, monocytes, macrophages, fibroblasts and epithelial cells [2,3]. It also has a substantial effect on regulating the function of neutrophils. IL-8 not only induces neutrophil adhesion to endothelial cells, but also stimulates chemotaxis and neutrophil granule exocytosis contributed by releasing lysosomal enzymes. Hence it is as an important factor in inducing inflammation and deterioration of periodontal diseases [2].Periodontal disease is regarded as a multifactorial condition that occurs as a consequence of interplay between environmental, behavioral, microbial and genetic factors. Genetic studies have revealed the polygenic nature of chronic periodontitis. Ten to twenty genes may be involved in such complicated multifactorial disease. These genes function differently in different ethnicities and populations and may result in the expression of different phenotypes under the influence of environmental factors. Certain syndrome types of periodontal diseases also exist, inherited in monogenic pattern [4].

The role of host genes in the etiology and pathogenesis of periodontal diseases are remarkably important to determine the risk and severity of tissue destruction. Genetic experiments may help to decide the susceptibility and recurrence of periodontitis and chances of tooth loss due to periodontal diseases. Hence considerable efforts have been done to conclude the role of genetic polymorphism as a risk factor in periodontal diseases. Due to the inherent complicated etiology of periodontal diseases, different genetic experiments may render varying results in different populations. Determining the predisposing genetic factors and specific associated inflammatory biomarkers may help the clinician to choose the most reasonable approach to prevent and control the periodontal condition in patients with high susceptibility of periodontal diseases [5].

While genetic susceptibility to different diseases differs in different populations and traits [4], this study was planned to investigate the association between IL-8 -251 (A/T) gene polymorphism and other clinical parameters to susceptibility of chronic periodontitis in patients referred to the dental school of Shiraz University of Medical Sciences, Shiraz, Iran.

MATHERIALS AND METHODS

This cross-sectional study planned enrolling 267 non smoking male and female individuals (227 periodontal patients and 40 healthy individuals) whilst all individuals were selected randomly, informed separately and signed the consent form. Specific clinical records including: number of existing teeth, Pocket Probing Depth (PPD), Clinical Attachment Level (CAL), O'Leary's Plaque Index (PI) and Bleeding on Probing (BOP) were registered [6,7].

Interproximal bone loss was also checked and observed for each patient on full mouth radiographs using the parallel technique with XCP (extension cone paralleling) device. The amount of bone loss was measured from CEJ to the level of the alveolar crest using a ruler then divided by the total root length and reported in percentage.

Those with no evidence of clinical attachment loss were categorized in healthy and considered as the control group.

The patient's periodontal status was categorized into three groups; namely, mild, moderate and severe conditions as defined below:

Mild: Maximum probing pocket depth of 3 mm and maximum mean bone loss of 15%

Moderate: Less than three interproximal sites with 50% or more bone loss and a mean bone loss between 16% and 34%

Severe: More than seven sites with 50% or more bone loss and a mean bone loss greater than 34% [8]

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Laboratory stages

A 7-8 ml volume of blood was obtained from each participant for DNA extraction. The

blood samples were mixed with 0.3 ml EDTA anticoagulant solution. DNAs were extracted via salting out method. In the salting out method, which is considered to be an easy and cost effective method, high concentrations of salts are used to produce genomic DNA in peripheral blood leukocytes. This method has the benefit of producing high quality DNA at a relatively high speed. Allel specific PCR (Polymerase Chain Reaction) technique was subsequently employed to determine the genotype of IL-8 on the 251st nucleotide. The primers wthat ere used in this study are shown below:

P1 : 5′-CCA CAA TTT GGT GAA TTA TCA AT-3′ for -251 A
P2 : 5′-CCA CAA TTT GGT GAA TTA TCA AA-3′ for -251 T
P3 : 5′-TCC CCC TTC ACT CTG TTA AC-3′

for common primer Two primers for β globulin gene were used as internal control to interpret Allele specific PCR (ASPCR):

P1 : 5′-ACA CAA CTG TGT TCA CTA GC-3′
P2: 5′-CAA CTT CAT CCA CGT TCA CC-3′

The test tubes were placed in the thermocyler (Indorf master cycler, Germany) for PCR procedures, so that after 5 minute preheating to 95° C, 30 cycles of denaturation, anneling and extension in appropriate temperature and time were done.The PCR product was then decanted into the cavities molded in 1.5% Agar gel to undergo electrophoresis (130 V, 30 Min) and then analyzed via UV transilluminator (UVp-Biodoc-II system, USA).

Statistical Analysis

To investigate the association between different genotypes of IL-8 and the severity of periodontal disease, and the association between different genotypes of IL-8 and clinical indices Kruskal-Wallis analysis and one-way ANOVA parametric test were used, respectively. Spearman's rho non parametric test and Pearson's correlation analysis were used to study the correlation between the patients' age and the severity of the disease, and between the patients' age and clinical indices, respectively. Man-Whitney test was used to reveal any association between gender and the severity of the disease and T test was used to show any correlation between gender and clinical parameters. To investigate the correlation between the number of teeth and clinical parameters, Pearson's correlation analysis was used. Tahman's test was also used to show any association between PI and BOP indices and the severity of the disease. Data were analyzed in SPSS software and the level of significance was determined as $P<0.05$.

RESULTS

The occurrence of genotypes between healthy subjects and patients with mild, moderate and severe periodontitis are summarized in Table 1.

Of the 227 patients with chronic periodontitis, 41 patients (18.1%) presented with AA genotype, 100 patients (44.5%) presented with AT genotype and 85 patients (37.4%) presented with TT genotype. In the control group, the following genotype frequencies were observed: 12 subjects (30%) with AA, 17 individuals (42.5%) with AT and 11 subjects (27.5%) with TT. Apparently, AT was the most frequent genotype among the cases and the control group (44.2%).

Kruskal-Wallis analysis failed to reveal any significant association between different genotypes of IL-8 and the severity of periodontal disease ($P= 0.164$).

Moreover, one-way ANOVA parametric test did not reveal any significant association between different genotypes of IL-8 and clinical indices of the mean PPD ($P= 0.525$), CAL ($P= 0.151$), bone loss ($P= 0.225$), PI ($P=0.087$), mean BOP ($P= 0.265$) and the number of remained teeth ($P=0.931$) (Table 2).

Table 1. Frequency of Genotypes Between Healthy Individuals and Patients with Mild, Moderate and Severe Periodontitis

	Control	Mild Periodontitis	Moderate Periodontitis	Severe Periodontitis
AA	12 (30%)	19 (21.3%)	13 (15.5%)	9 (16.7%)
AT	17 (42.5%)	39 (43.8%)	39 (46.4%)	23 (42.6%)
TT	11 (27.5%)	31 (34.8%)	32 (40.7%)	22 (27.5%)
Total	40	89	84	54

Table 2. Association Between Genotypes of IL-8 and Clinical Parameters

	AA	AT	TT	P value
PPD	2.75± 1.16	2.93± 1.01	2.95±1.1	0.525
CAL	2.99± 1.3	3.39± 1.32	3.4±1.37	0.151
Bone loss	17.86± 15.39	20.592± 14.71	22.11± 15.04	0.225
PI	62.7± 32.89	60.75± 31.28	69.98± 29.24	0.087
BOP	55.32± 36.61	56.75± 32.67	63.02± 30.18	0.265
Number of teeth	26.25± 4.95	26.35± 4.13	26.12± 4.50	0.931

Table 3. Correlation Between Gender and the Clinical Parameters

	Male	Female	P value
Number of teeth	26.3± 4.7	26.2± 4.3	0.82
PPD	2.73± 1.08	2.98± 1.07	0.082
CAL	3.23± 1.48	3.35± 1.28	0.508
BL	19.46± 16.37	21.08± 14.32	0.412
PI	56.7± 34.70	66.67± 29.05	0.107
BOP	54.13± 35.87	60.86± 30.94	0.138

Furthermore, Spearman's rho non parametric test revealed a weak, but significant correlation between the patients' age and the severity of the disease (r =0.139, P=0.024) and Pearson's correlation analysis showed a significant reverse correlation between the number of teeth and the patient's age (r= -0.316, P<0.001). Age was also significantly related with the other clinical indices at different correlation coefficients. PPD (r=0.196, P=0.001), PI (r=0.171, P=0.005) and BOP (r=0.178, P=0.003) showed a weak correlation whilst CAL (r=0.315, P<0.001) and BL (r=0.344, P<0.001) were moderately correlated.

Statistical test of Man-Whitney revealed that male individuals were significantly more prone to severe periodontitis (P<0.001). T test however, failed to show any significant correlation between gender and clinical parameters (Table 3). Further statistical analysis rendered weak reverse correlation between the number of teeth and probing depth (r= -0.234), and a moderate reverse correlation between the number of teeth and CAL (r=-0.348), bone level (r= -0.392), Plaque Index and BOP (r=-0.33), all of which were significantly relevant (P<0.001). Bone loss was moderately correlated with PI and BOP (r=0.6, P<0.001). CAL and PPD were similarly correlated with PI and BOP (r=0.6, P<0.001). Furthermore, ANOVA statistical test revealed significant correlation between the severity of the periodontal condition and plaque index and BOP (P<0.001).

Tahman's statistical test failed to signify any substantial difference of PI and BOP indices between patients with moderate and severe periodontitis; while they were significantly different in patients with mild periodontitis and the control group.

DISCUSSION

IL-8 -251 (A/T) single nucleotide polymorphism affects the function of promoter gene and cases presenting with AA genotype produce and release higher levels of IL-8 compared to individuals with AT or TT genotype. Hence, this type of polymorphism is considered as a rational source of investigation to study the role of genetic factors in the incidence and severity of inflammatory diseases [9].Bleeding on probing is an important index for diagnosis and evaluation of treatment in periodontal inflammation. It can also be used as an important tool to inform patients of their current condition. In the present study, we failed to observe any significant differences in BOP between different genotypes of IL-8 (P=0.265). This was in line with studies conducted by Shimada et al. and Scapoli et al. who also found no significant association between BOP and genetic polymorphism of TNF α, β and IL-1α, respectively [8,10]. Lang *et al.* [11], Muller *et al.* [12] and De Sanctis *et al.* [13] however, reported a positive correlation between IL-1 gene polymorphism and BOP.

Pocket probing depth, clinical attachment level and bone loss are the major indices that help diagnose the onset and severity of periodontal disease. We failed to reveal any significant correlation between IL-8 gene polymorphism and clinical parameters associated with periodontitis among the Shirazian population. Although Kornman *et al.* [14], McDevitt *et al.* [15] and Galbraith *et al.* [16] documented possible correlation between IL-1α and IL-1β gene polymorphism and periodontitis, our findings were in concordance with Mark *et al.* [17] and Laine *et al.* [18]. This study suggests that although IL-8 is an important cytokine in the occurrence of periodontitis, 251-(A/T) IL-8 gene polymorphism does not appear to influence the severity of periodontitis. In two studies on a Brazilian population, the authors failed to demonstrate any association between 353-(A/T) IL-8 gene polymorphism and the susceptibility to periodontal disease [19] and also failed to reveal any association between single-nucleotide polymorphism (rs4073) in IL-8 gene promoter region and susceptibility to periodontitis in Brazilian individuals [20]

that seems to be in line with the results of the present study. In contrast, Andia et al. showed single nucleotide polymorphism (SNP) reference sequence 4073 IL-8 gene promoter was associated with chronic periodontitis in non-smoker Brazilian subjects and the frequency of the A allele was higher in the disease group [21]. In addition, another recent study showed an association between the other genotype +396 (T/T) and chronic periodontitis susceptibility in Brazilians [22]. Kim *et al.* suggested that although -845 (T/C) and -738 (T/A) IL-8 gene polymorphisms were not individually associated with periodontitis, some haplotypes showed significant association with susceptibility to, or protection against, chronic periodontitis in a Brazilian population [23].

Whether age can be encountered to have any association with the condition or not has been a dilemma for a long time between researchers. Although evidence has refuted the direct relation between age, as a sole factor, and the severity of the condition, the cumulative effect of predisposing factors over time has been proved to increase the chances of severe periodontitis. In the present study, the average age range of patients with severe periodontitis was significantly greater than that of mild and moderate periodontitis. According to this data, severe periodontitis is more likely to occur in older patients. Although our results failed to show any significant correlation between gender and the clinical indices of periodontitis, male individuals are acquainted with the more severe form of the disease. This was consistent with Li's study that reported the collective effect of gender and genotype in the occurrence of periodontitis [24].

According to their study, males with A allele were more prone to chronic periodontitis and on the contrary, presence of G allele is associated with a lower chance of periodontitis [24]. Our results indicate that there is no definite correlation between 251(A/T) IL-8 gene polymorphism and the severity and propensity to periodontal diseases.

CONCLUSION

Within the limitations of this study, no significant correlation was observed between gene polymorphism and the severity of periodontal disease in the selected Iranian population. Although there were differences in the frequency of AA genotype between the cases and the control group, it was not statistically significant.

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