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IL-1β_{+3954} Genetic Polymorphism Association with Generalized Aggressive Periodontitis in Khorasan-Iran Province

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The aim of this study was to investigate the relationship between IL-1β_{+3954} gene polymorphism and Generalized Aggressive Periodontitis (GAP). This study included 25 patients diagnosed as GAP from Khorasan province and 18 periodontally healthy controls. They were referred to Periodontology Department of Mashad Dental School. Extracted DNA from peripheral blood was evaluated by PCR-RFLP method. Data were analyzed using Chi-square test, Fisher's exact test and two sample t-tests. There was no significant association between IL-1β_{+3954} genotype and GAP disease ($p = 0.419$). There was no significant association between IL-1β_{+3954} alleles and GAP disease either ($p = 0.370$). However, there was some trend for greater of 1.1 genotype and allele 1 prevalence among GAP patients compared to controls (56 and 44.4% for 1.1 genotype among GAP and control patients respectively, 78 and 69.4% for allele 1 frequency among GAP and controls, respectively). The frequencies of 1.1 genotype and allele 1 in the population of this study were 51.2 and 74.4%, respectively. The lack of any association between IL-1β_{+3954} polymorphism and GAP, in the population presented here, brings into doubt the usefulness of this gene as a marker of susceptibility to GAP. To elucidate this relationship, more research is necessary.

Key words: IL-1, gene polymorphism, periodontitis

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INTRODUCTION

Periodontal disease is an inflammatory disease that represents the main cause of tooth loss in developed countries, with increasing prevalence in the developing world (Albandar and Rams, 2002). Although the presence of gram-negative bacteria is essential for initiating and perpetuating periodontal disease, environmental as well as genetic factors contribute to individual variations in the etiology and course of disease (McDevitt *et al*., 2000).

Individual differences in periodontal disease progression and treatment outcomes have long been observed (L¨oe *et al*., 1978; Lindhe *et al*., 1983; Lindhe and Nyman, 1984). Disease initiation is believed to be caused by bacteria (Socransky and Haffajee, 1992, 1994), but individual differences in the inflammatory and immune response to infection may affect the susceptibility to the disease (Page *et al*., 1997).

Inflammation is a complex process that develops following initial tissue trauma and is completed with induction of tissue repair. The innate immune system is of central importance to the initiation, progression and containment of the inflammatory response (Takashiba and Naruishi, 2006). Cytokines are potent immunomodulatory molecules that act as mediators in immune response pathways. The pro-inflammatory cytokines IL-1β and TNF-α mediate inflammatory responses by attracting and activating, white blood cells to tissues and stimulating the secretion of other lymphocytotropic cytokines and catabolic enzymes. Interleukin-1β is a proinflammatory cytokine produced by monocytes, macrophages and epithelial cells (Socransky and Haffajee, 1992). This cytokine is a primary activator of early chemotactic cytokines, as well as of the expression of adhesion molecules that facilitate migration of leukocytes into tissues. IL-1 is also known to be one of the most active stimulators of osteoclastic bone resorption (Lang *et al*., 2000).

Interleukin-1β activity is modulated by an endogenous factor, interleukin-1 receptor antagonist (Dinarello, 1998). By binding to the interleukin-1β receptor without exerting an effector function, interleukin-1 receptor antagonist acts as a competitive antagonist for interleukin-1β (Arend, 1991). The role of IL-1β has been studied extensively in periodontal diseases (Eisenberg *et al*., 1991). IL-1 levels have been reported to be elevated in periodontal tissues and gingival cervicular fluid associated with periodontitis. Genetic variations that disrupt innate immune sensing of tissue injury could explain individual differences in the ability of the immune system to respond to tissue injury, the diversity of the clinical presentation of inflammation and the response to current medical treatment (Barton and John, 2003).

Several cytokine genes harbor genetic polymorphisms (Duff, 1993) that may influence the level of cytokine secretion and may therefore explain the individual differences in the cytokine responses to bacterial stimuli (Kjeldsen *et al*., 1995; Kornman and di Giovine, 1998).

There are three genes that regulate the production of IL-1: IL1A, IL1B and IL1RN (Nicklin *et al*., 1994). These genes are located on chromosome 2q13. Genes IL1A and IL1B control the production of the pro-inflammatory proteins, IL-1α and IL-1β, respectively. IL1RN controls the synthesis of an antagonist protein (IL-ra) (Greenstein and Hart, 2002). Polymorphism in the +3954 locus (formerly designated as +3953), of the IL1B gene has been associated with an increased production of this cytokine. Homozygous individuals for the T allele produce a four-fold higher amount of IL-1β compared to individuals displaying the CC genotype (Pociot *et al*., 1992). Polymorphisms within the IL-1β gene are associated with altered protein production rates and certain haplotypes have been linked to certain types of periodontitis (Kornman *et al*., 1997; Gore *et al*., 1998; Diehl *et al*., 1999; Galbraith *et al*., 1999; Mc Devitt *et al*., 2000; Parkhill *et al*., 2000; Papapanou *et al*., 2001).

It has recently been suggested that this polymorphism may explain why some people have a more vigorous response than others to the same stimulus (Lang *et al*., 2000). Several studies have evaluated gene polymorphisms in individuals with periodontitis in distinct populations. Kornman *et al*. (1997) demonstrated that the simultaneous occurrence of IL1A (-889) and IL1B (+3954) polymorphisms were associated with a severity of chronic periodontitis in non-smoker Caucasians. Walker *et al*. (2000) observed a high prevalence of IL1B (+3954) allele C in the African- American population and concluded that this polymorphism would provide little diagnostic or predictive information for localized aggressive periodontitis. Moreira *et al*. (2005) suggested that the polymorphism in the locus +3954 of IL1B gene could be a risk factor for chronic periodontitis in a sample of Brazilian individuals.

Jepsen *et al*. (2003) failed to demonstrate an association of the IL1 genotype to the rate of development of GCF volumes and percentage BOP in experimental gingivitis

The distribution of gene polymorphisms varies among different ethnic groups. Since genetic susceptibility to disease most likely is influenced by a multitude of genetic factors, it is most likely that the impact of a single gene polymorphism on disease susceptibility also varies among ethnical and racial groups. To date, there has been no study on the inter-relationship between IL $1\beta_{+3954}$ gene polymorphism and

generalized aggressive periodontitis in Persian population. Therefore, the aim of this study was to investigate the association of the IL $1\beta_{+3954}$ (C/T) gene polymorphism with aggressive periodontitis in Khorsan-Iran province.

MATERIALS AND METHODS

Study population: This study was conducted at the Deparment of Periodontology, Mashhad Dental School and Bu-Ali Research Institute, Masshad University of Medical Sciences in 2005. The study protocol was approved by the ethical committee of the University. In this study a total of fifty unrelated, nonsmoking Iranian-Khorasanian (North-East of Iran) subjects <30 years of age were selected. The diagnosis of periodontitis was based on physical examination, medical and dental history, probing depth, assessment of attachment loss, tooth mobility and radiographs. Twenty four patients (18 females, 6 males) were affected by generalized aggressive periodontitis and the remaining twenty six subjects (16 female, 10 male) were periodontally normal. No systemic disorders were present in either groups and none of the individuals was smoker. Written informed consent was obtained from participants.

Genotype identification: Genomic DNA was isolated from whole blood collected with EDTA as anticoagulant, using a salting out method with commercial Biogene kit (Mashhad-Iran). A specific portion in exon V at position +3954 of IL-1β gene was amplified by Polymerase Chain Reaction (PCR). Specific primer sequences (Sense; 5'-GTTGTCATCAGACTTTGACC-3' and Antisense; 5'-TTCAGTTCATATGGACCAGA-3') were derived from the original sequence. The PCR was performed in 20 μL reaction, containing 100-200 ng DNA, 500 μmol of specific primers, 0.5 unit Taq DNA polymerase, 10x PCR reaction buffer (100 mM L^{-1} Tris-HCl, 500 mM L^{-1} KCl, 15 mM L^{-1} $MgCl_2$), 200 μM each dNTP. The Amplification was carried out using a thermocycler under the following conditions: Program 1, for 2 cycle: 2 min at 95°C, 1 min at 68°C, 1 min at 72°C; Program 2, for 35 cycle: 2 min at 95°C, 1 min at 60°C, 1 min at 72°C and program 3 for 1 cycle: 1 min at 94°C, 1 min at 68°C and 5 min at 72°C. Corbet thermocycler (Corbet Research, Australia) was applied. PCR products then were digested by restriction enzyme Taq1 (20 U mL^{-1}) (Fermentas, Germany) for 3 h and cleaved DNA fragments were subjected to electrophoresis in 17% polyacrylamide gel and stained with silver nitrate. The presence of 2 size bands of 112 bp and 138 bp indicated that a 1.1 genotype occurred. The visualization of a 250 bp band indicated that a 2.2 genotype existed. Finally the presence of all 3 above mentioned bands indicated the presence of a 1.2 genotype.

For the GAP patients, clinical periodontal parameters were recorded. Probing pocket depth at six sites around each tooth was measured using a Williams' probe. Bleeding on probing as well as gingival index (Loe and Silness, 1963) were also measured and recorded.

Data analysis: The significance in allelic frequencies between control subjects and periodontitis patients were tested using Fisher's exact and Chi-square tests. The mean probing pocket depth, percentage of pockets ≥5 mm and percentage of BOP positive sites as well as mean gingival index were compared between the 1.2 and 1.1 genotypes across the GAP patients using the 2 sample t-test. The data analysis was accomplished using SPSS ver. 10 software.

RESULTS

There was no significant association between IL-$1\beta_{+3954}$ genotype and GAP disease (p = 0.418) (Table 1). We found no significant association between IL-$1\beta_{+3954}$ alleles and GAP disease either (p = 0.370). However, there was some trend for composite 1.1 genotype and allele 1 prevalence in GAP patients compared to controls (56 and 44.4%, respectively for 1.1 genotype and 78 and 69.4%, respectively for allele 1). The frequencies of 1.1 genotype and allele 1 in the population of this study were 51.2 and 74.4%, respectively. Using the hypothesis that the 1.1/1.2 composite genotype is more prevalent in GAP, there is no significant difference in GAP cases and controls. However, in GAP cases showed more 1.1/1.2 composite genotype. No significant difference detected between 1.1 and 1.2/2.2 composite genotype (p = 0.455) (Table 2). There were not any significant differences in clinical parameters between the two genotype (Table 3).

Table 1: IL-β genotype and allele frequencies in Healthy Control (HC) individuals and in patients with GAPS

	Genotype			Allele frequencies (%)	
	1.1	1.2	2.2	1	2
HC (n = 18)	8	9	1	25	11
GAP (n = 25)	14	11	0	39	11

Table 2: Cmparison between two genotypes (1.1 or 1.2 with 2.2) in Healthy Control (HC) individuals and in patients with GAP

Genotypes	GAP (n = 25)	HC (n = 18)	Total	p-value
1.1 or 1.2	25 (59.9%)	17 (40.5%)	42 (97.7%)	0.419
2.2	0 (0.0%)	1 (100.0%)	1 (2..3%)	
1.1	14 (63.6%)	8(36.4%)	22(97.7%)	0.455
1.2 or 2.2	11(52.4%)	10(47.6%)	21(2.3%)	

Table 3: Comparison between clinical parameters in 1.1 and 2.2 genotypes

Genotype		No.	Mean	SD	SEM	p-value
Mean PD	1.1	14	3.8436	0.64200	0.171600	0.538
	1.2	11	3.7136	0.38720	0.116700	
Mean GI	1.1	14	1.5636	0.21110	0.056420	0.858
	1.2	11	1.5464	0.26400	0.179600	
% Bop	1.1	14	0.9836	0.03272	0.008764	0.589
	1.2	11	0.9709	0.07867	0.023720	
% PD≥4	1.1	14	0.4464	0.09779	0.026140	0.758
	1.2	11	0.4591	0.10480	0.031610	
% PD≥5	1.1	14	0.3486	0.12410	0.033160	0.958
	1.2	11	0.3464	0.08262	0.024910	

DISCUSSION

Aggressive periodontitis is an infectious and inflammatory process which leads to periodontal attachment loss and exfoliation of the teeth. The progression of the disease is influenced by several factors including bacterial plaque, genetic predisposition, environment-gene interaction, etc. (Hart and Kornman, 1997; Salvi *et al*., 1997). It is increasingly evident that genetic variance is a major determinant of the differential risk for many human diseases including periodontitis. Genetic alteration could change the transcript level of the proteins. The result can range along continuum of functional consequences from no observable change in protein function to a minor change in function, a dramatic change or obliteration of function (Kinane *et al*., 2005).

A great proportion of genetic background of GAP is still unidentified and only few cytokine gene polymorphisms have been studied (Soafer, 1990; Hart, 1996). IL-1 is the main pro-inflammatory factor in the pathogenesis of GAP and the members of its gene family (located on the long arm of chromosome 2) are reasonable candidates to evaluate their contribution of risk in GAP.

In the present study, we chose IL-1β_{+3954} coding IL-1 proteins to analyze any association between particular alleles and susceptibility to GAP. This study was the second of such studies to investigate association of gene polymorphism with GAP in Iranian Khorasanian population. Recently we demonstrated that subject with C/C genotype of TGF-β_1 polymorphism at -509 position might be more prone to the risk of developing aggressive periodontitis as compared to other genotypes (Arab *et al*., 2006). In the present study we observed no significant association between GAP and IL-1β_{+3954} gene polymorphism however there was some trend for 1.1 genotype and allele 1 prevalence in patients compared to controls. Several investigations revealed that there was association between allele 2 and Chronic Periodontitis (CP), whereas allele 1 related to aggressive periodontitis. Considering these studies and results we concluded that CP and GAP differ in nature. Patients with allele 1 are susceptible to acquire disease in adolescence, whereas individuals with allele 2 may get the disease only at their adulthood stage of life. In this study, lack of any association between periodontitis and IL-1β_{+3954} gene shows that possibly more than one gene polymorphism might be involved in the susceptibility to disease, that is a composite genotype including of more than one gene should be studied rather than a single gene polymorphism such as IL-1β_{+3954}. Furthermore an unveiled interaction exists between the IL-1 genetic polymorphism and environmental factors such as smoking (Meisel *et al*., 2004). Smokers bearing the genotype-positive IL-1 allele combination may be at an increased risk of developing periodontitis. This suggests that genetic-environmental interaction is more important than genetic factors alone for determination of susceptibility to periodontitis. The patients of our study were deliberately selected from non-smoker to prevent confounding results by such interaction.

Different IL-1 polymorphisms have been associated with periodontitis (Deihl *et al*., 1999) and with periodontitis associated with cardiovascular disease (Kornman *et al*., 1999; Kornman and Duff, 2001). While the composite allele 2 polymorphism has been associated with increased IL-1β production by activated peripheral blood neutrophils isolated for patients with advanced disease (Gore *et al*., 1998), the relationship between IL-1 polymorphisms and disease progression remains to be determined. Indeed, Diehl *et al*. (1999) have suggested that the composite allele 2 polymorphism which gives rise to high level of IL-1β may, in fact, be protective for periodontitis.

Our results also showed that there was not any association between IL-1β_{+3954} genotype and clinical parameter including mean pocket depth, percentage of sites with bleeding on probing, mean gingival indices, percentage of pockets deeper than 4 and 5 mm. This finding of our study is agreement with those of Papapanou *et al*. (2001) and Meisel *et al*. (2002) who reported no associated between IL-1 composite genotype and clinical parameters such as probing depths, bleeding on probing and plaque. Collectively the results of present showing lack of any association between IL-1β_{+3954} polymorphism and GAP in the population presented here brings into doubt the usefulness of this gene as a marker of susceptibility to GAP. To elucidate this relationship, more research is necessary.

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