Genetic association of IL-8 (rs4073) gene polymorphism with susceptibility to chronic periodontitis: A case control study in south Indian population.

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Abstract

Many environmental and bacterial factors are in the initiation and modulation of periodontal disease. Recent studies show increasing evidence that genes also play a vital role in the etiology and progression of periodontal diseases. Interleukin 8 is a neutrophil chemotactic agent that mediates the inflammatory reaction in periodontal disorders. The present study was done to analyse the association of IL8 polymorphism with periodontitis patients.

Keywords: Chronic periodontitis, Genetic study, IL8, Gene polymorphism, Disease susceptibility, Innovative study.

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Introduction

Periodontitis is the chronic inflammation of the periodontium which involves the interaction between bacterial colonies, cell populations of the oral mucosa, inflammatory mediators and host susceptibility. The activation of the host defence cells is initiated by substances released from the biofilm on the teeth surface which include lipopolysaccharides, antigens and other virulence factors [1]. The pathogenesis of periodontitis is linked with a dysregulated host inflammatory-immune response to intra-oral plaque bacteria [2]. While it has been proved that environmental and bacterial factors initiate and modulate the progression of periodontal disease, there now exists evidence supporting the theory that genes play a vital role in the etiology and progression of periodontal diseases [3–8]. Cytokines play an important role in deterioration of gingival tissue in periodontal diseases, and genetic polymorphisms which occur within these genes are considered to be key inducers of these periodontal diseases [9-10].

One of the factors of interest in periodontal diseases is Interleukin-8 (IL-8), a powerful neutrophil chemotactic agent. IL8 protein which is encoded by the IL8 gene, located on the chromosome 4q12q13 and belongs to the superfamily of CXC chemokines, is a potent chemokine responsible for inducing chemotaxis which is the migration of the inflammatory cells such as neutrophils, T cells, and basophils to the site of inflammation. It however, does not lead to activation of monocytes. IL8 binds to CXCR1 receptor on the neutrophils which leads to activation of the neutrophils, thus initiating an immune response [11]. This response is mediated by a G-protein receptor that activates a phosphatidylinositol-calcium second messenger system. This special coordinated expression of IL-8 allows the movement of neutrophils from the highly vascularized gingival tissue to the gingival crevice [12]. IL8 produces hyperalgesia and is unique due to the fact that it is produced early in the stages of inflammatory response while its presence lasts for a prolonged duration of time. Many studies have shown there is increased expression of IL8 in affected periodontal tissues. Studies have been undertaken to check the IL8 levels in the GCF and saliva and many have contradicting results [13]. While some studies show high levels of IL8 in GCF and saliva of periodontitis patients [14,15] , others show the opposite results [16,17].

The genetic factors for periodontitis has been extensively researched in past studies. Studies have been done to identify specific genes in association with periodontitis. A polymorphism of the IL8 gene promoter region at the position 251 T/A (rs4073) has been associated with many diseases including breast cancer, prostate cancer, bronchiolitis, lung cancer, macular degeneration and coronary artery disease. As a result of the inherent complicated etiology of periodontal diseases, varying results of genetic experiments can be obtained from different populations. Research based on the association of IL8 polymorphism and periodontitis has been performed on populations including Indonesian, Brazilian, Chinese, Iran and Czech Republic. Therefore, it would be interesting to investigate the association of the 251 T/A polymorphism (rs4073) with periodontitis in the South Indian population. Our team has extensive knowledge and research experience that has translate into high quality publications [18–37]. The intent of this study was to investigate the association of the IL8 rs4073 polymorphism with generalized periodontitis in families and unrelated individuals with and without periodontitis from the South Indian population [38,39].

Materials and Methods

This study employed a cross-sectional design involving individuals from Chennai, Tamil Nadu, India. A total of 100 individuals who reported to the Department of Periodontics, Saveetha Dental College, Chennai, were included in this study. The subjects were divided into a control group A (N=50) and CP group B (N=50) based on the clinical examination of probing pocket depth, clinical attachment loss and bleeding on probing. The CP group contained 50 patients of which 26 were males and 24 were females with a mean age of 39.02 ± 8.22 years. The CP patients were recruited based on the 1999 criteria of the American Academy of Periodontology. The control group contained 50 periodontally healthy subjects (26 male, 24 female) with mean age of 41.34 ± 7.49 years. A

detailed history of dental treatment, family history of periodontal diseases, smoking habits as well as general health concerns were obtained from the subjects. Except for periodontitis, the subjects were clinically healthy. Smokers, pregnant or lactating mothers, immunocompromised individuals and subjects who had undergone periodontal therapy within the past 6 months were excluded from this study.

Sample collection and DNA extraction

A volume of 5 mL of venous blood was collected from the antecubital fossa and dispersed in a sterile tube containing a pinch of ethylenediaminetetraacetic acid. It was mixed thoroughly to avoid clot formation. The isolation of DNA was performed in accordance to the modified Miller et al. protocol.

Polymerase chain reaction and restriction endonuclease digestion

IL-8 receptor gene polymorphisms were assessed by Polymerase Chain Reaction (PCR) amplification and restriction digestion. The primers forward 5′-CCATCATGATAGCATCTGTA-3′ and reverse 5′-TGGCTCTTGTCCTAGAAG-3′ were used for amplification of DNA. The DNA amplification was done using 10 mg of genomic DNA, 5 pmol/µL of both forward and reverse primers together with PCR Master Mix (Takara, Shiga, Japan) in 20-µL volumes. The cycling conditions were as follows: initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 35 seconds, annealing at 60°C for 35 seconds, followed by 35 seconds at 72°C and finally at 72°C for 5 minutes. A 5-µL volume of PCR product was checked on a 1% agarose gel, and 15 µL of PCR product was digested using a MfeI restriction enzyme (New England Biolabs, Hitchin, UK). Digestion was carried out at 37°C for 2 hours. The digested product was visualized on 2% agarose gel and the results were documented.

Statistical analysis

The statistical analysis was done using SPSS version 23.0 (SPSS, Chicago, IL, USA). The $\chi 2$ test was used to compare the distribution of genotypes and allele frequencies in the periodontitis and control groups. The risk associated with individual alleles or genotypes was calculated as the Odds Ratio (OR) with 95% confidence intervals. Statistical significance in all tests was set at P<.05.

Results

Results indicate there was no evidence of deviation from Hardy-Weinberg equilibrium (p>0.05). Study results show that genotype frequency of VDR MfeI polymorphism did not differ significantly at $\chi 2$df (P=0.257). The prevalence of homozygous and heterozygous mutant genotypes had no significant difference (AA *vs.* AT+TT) between the periodontitis and healthy control group with a P-value of 0.1098. The genotypes frequencies in the control group with TT=44%, AT=38% and AA=18% and the case group with TT=34%, AT=34% and AA=32% was observed. The detected frequency of AT (34% *vs.* 38%), and TT (34% *vs.* 44%) genotypes showed no significant difference between the periodontitis group and healthy control subjects. However, the AA genotype was seen more in the case population when compared to the control (32% *vs.* 18%). There was no significant difference in A allele (49% *vs.* 37%) and T allele (51% *vs.* 63%) between the CP and healthy control group (Figures 1 and 2; Tables 1 and 2).

Groups	AA	AT	TT	A	T	HWE (p value)*
Case (N=50)	16	17	17	0.49	0.51	0.0237*
Control (N=50)	9	19	22	0.37	0.63	0.1910*

Table 1: *Genotype frequencies of IL-8 (rs4073) gene polymorphism among the cases and controls. *For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom. Deviation from HWE was observed in cases*. The genotype frequency of cases and controls do not differ significantly/2df (P=0.257).*

Dominant				
Genotypes	**Case**	**Control**	**Unadjusted OR (95% CI)**	**P value**
AA	16	9	2.1438 (0.8419-5.4590)	098
AT+TT	34	41		
Recessive				
AT+AA	33	28	252 (6793-3.4245)	063
TT	17	22		
Allele				
A	49	37	395 (9304-2.8766)	874
T	51	63		

Table 2: *Overall genotype distribution of the IL-8 (rs4073) gene polymorphism in cases and controls.*

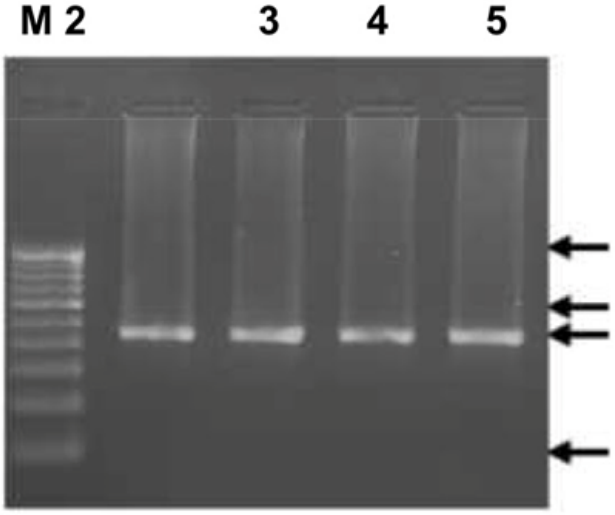

Figure 1: *Agarose gel electrophoretogram of IL-8 (rs4073) showing 462 bp amplicon in lanes 2-5 (Lanes 1 (M):100 bp DNA ladder).*

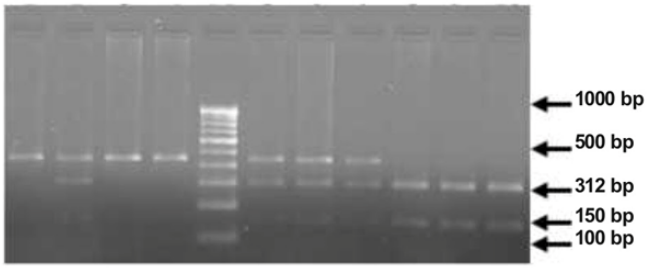

Figure 2: *Mfel digestion of PCR amplified product (Lanes 1, 3, 4 -TT Wild type, 2,5,6,7-AT - Heterozygous, 3-AA - Homozygous, Lane 5-100 bp DNA ladder).*

Discussion

In the present study we analysed the association patterns of IL8 (rs4073) with periodontitis using case–control study designs. Genetic polymorphisms along with environmental factors and other genetic predispositions aid in increasing the susceptibility of an individual to a specific disease. Many previous studies reveal that single nucleotide polymorphisms play a role in the pathogenesis of periodontitis. As the immune system plays a crucial role in the pathogenesis of periodontitis, there have been studies on the identification of genetic polymorphisms in multiple aspects of immunity. Allelic variants at multiple gene loci tend to influence susceptibility to periodontitis. IL8-251 (A/T) SNP affects the function of promoter gene and cases presenting with the AA genotype produce and release higher levels of IL-8 when compared to individuals with TT or AT genotype 39.

Our study results show that genotype frequency of VDR MfeI polymorphism did not differ significantly at χ2df (P=0.257). The prevalence of homozygous and heterozygous mutant genotypes showed no significant difference (AA *vs.* AT+TT) between the periodontitis group and healthy control group with a P-value of 0.1098. The detected frequency of AT (34% *vs.* 38%), and TT (34% *vs.* 44%) genotypes showed no significant difference between the periodontitis group and healthy control subjects. However, the AA genotype was seen more in the case population when compared to the control (32% *vs.* 18%).

A few previous studies have been reported on the IL8 251 A/T gene polymorphism and periodontitis [40–42]. Recent studies in Brazilian population with the same IL8 polymorphism, reported lack of association of the polymorphism with periodontitis.

In a subsequent study, when the same gene polymorphism was investigated as part of combined haplotypes (−845TC, −738TA and −353AT), it was reported that nonsmoking individuals presenting with the TAT/CTA genotype were six times more susceptible to periodontitis.

The present study also reported lack of association with periodontitis. As a future prospective, the investigation of the IL8 251 A/T gene polymorphism as part of haplotypes might throw light into some new perspectives in the South Indian population.

In another study of the association between the IL8 polymorphism and generalized aggressive periodontitis, a frequency of 65.8% of the TA genotype was seen in cases which was higher than that in control [43]. However, such an association was not reflected in the current study with a frequency of 34% in case and 38% in control. Therefore, a lower frequency of the TA genotype was found in the aggressive periodontitis patients, compared to the chronic patients. This fact leads us to think about the possible differences between the genetic profiles of chronic and aggressive periodontitis.

The statistical power was not sufficient in the present study due to smaller sample size and lesser diversity in population. There is the possibility of a false negative result and careful analysis has to be done to state that there is no association between the IL8 251 A/T gene polymorphism and periodontitis in the South Indian population.

Further studies can be done to increase our understanding of IL-8 in the etiology of periodontitis and provide better treatment plans. Our institution is passionate about high quality evidence based research and has excelled in various fields [44–48]

Conclusion

The present study showed no significant association between IL8 251A/T gene polymorphism and periodontitis patients. However, larger and better-designed studies are needed to validate our findings.

In addition, gene-gene and gene-environment interactions have to be considered, to ensure a better, more comprehensive understanding of the association between IL-8 polymorphisms and periodontitis susceptibility.

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Conflict of Interest

The authors declare no conflict of interest.

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