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Association Of Il18 Gene Polymorphism with Susceptibility to Periodontitis - A Case Control Study

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ABSTRACT

Background: Cytokine IL18, also known as IFN-inducing factor, is an important regulator of innate and acquired immune responses and plays a number of roles in chronic inflammation and dysfunction autoimmune disorder. Several polymorphisms in the IL18 promoter region have been identified. As there is little evidence for IL18 polymorphisms and periodontitis, this study aimed to evaluate the genetic association of polymorphisms of the IL18 gene (rs1946518).
Materials and Methods: A total of 100 subjects were recruited for this study, including 50 patients with periodontitis (stage II, III and grade B) and 50 patients with healthy gingiva clinically intact periodontium. Genomic DNA was extracted from the blood obtained from the subjects. DNA was amplified using primers specific to the MseI region of the IL18 receptor gene. Amplicon was further genotyped using the restriction fragment length using the BtgI enzyme. Genotyping obtained on the basis of the RFLP model was recorded and used for statistical analysis. The distributions of genotypes and allele frequencies in the periodontal disease group and the control group were compared using the Chi Square test. Risks associated with individual alleles or genotypes were calculated using odds ratios (ORs) with 95% confidence intervals. Statistical significance in all trials was determined at $p < 0.05$.
Result: The frequencies of genotypes and the distribution of IL18 receptor MseI polymorphisms were not significantly different at $\chi 2df$ (P = .818). The results of our study showed that there was no significant difference between homozygous and heterozygous genotypes (GG vs TG TT) between periodontitis patients and control group with a P value of 0.8316. The detection frequencies of the TG (20% vs 22%) and TT (14% vs 10%) genotypes showed a significant difference (P value = 0.8316) between the control and experimental groups. experience. There were no significant differences for the G allele (76% vs 79%) and the T allele (24% vs 21%) between the experimental and control groups.
Conclusions: The present study indicated that the polymorphism of the IL18 gene was not associated with CP in the analyzed study group.

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INTRODUCTION

Periodontitis is a chronic inflammatory disease, influenced by multiple factors that caused the destruction of the supporting tissues and resulted in tooth loss[1,2,3,4]. The risk factors of periodontitis can be modifiable risk factors like Diabetes mellitus, smoking where genetics is a non-modifiable risk factor that influences a person's vulnerability to periodontal disease[5]. The proinflammatory cytokines formed by host-derived periodontal tissue (e. g. IL-1, IL-6, TNFα) contribute to the degradation of the periodontal structural components that lead to clinical symptoms of periodontitis. The classical interleukin-1 (IL-1) family cytokines, IL-1a and IL-1b, as well as IL-18, play key roles in the development of periodontal disease [6,7,8].

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The cytokine IL-18, also known as IFN–inducing factor, is a significant regulator of innate and acquired immune responses and plays several roles in chronic inflammation and autoimmune disorders. IL-18 is a pleiotropic cytokine that is found at moderately high levels in a variety of cell types including macrophages, osteoblasts, monocytes, macrophages, keratinocytes, intestinal epithelial cells, astrocytes, dendritic cells, and microglia[9,10]. IL-18 is a glycoprotein with a molecular weight of 18 kDa that is synthesised as a 23 kDa inactive precursor (pro-IL-18) that must be cleaved by caspase-1 to become an activated cytokine [10,11]. Based on its ability to facilitate the secretion of other pro-inflammatory cytokines such as TNF, IL-1, IL-8, and GM-CSF, IL-18 has good pro-inflammatory effects, enhancing neutrophil expansion, migration, and activation during infections [12]. Its expression may be stimulated and increased in response to infectious and inflammatory stimuli such as LPS, gram-positive bacteria exotoxins, or other cytokines (e.g. IL-1, TNF, IL-6, IL-10)[13,14]. In rheumatoid arthritis, IL-18 has been shown to play a role in the deterioration of bone and cartilage, favouring the growth of osteoclasts and bone resorption[15,16].

The influence of IL-18 on MMP expression in periodontitis has been studied intensively. Previous studies have shown that binding of IL-18 to the IL-18 receptor activates nuclear factor-B (NF-B)[17] and this NF-B, a pleiotropic transcription factor controls the expression of several genes involved in immune and inflammatory responses. The NF-B signalling pathway is activated by a variety of stimuli, including bacterial or viral infections, ionising radiation, and inflammatory cytokines. To promote transcription, activated NF-B translocates to the nucleus and binds to target genes and is involved in cell proliferation, apoptosis, inflammation, and immunity[18]. The activation of NF-κB increases the expression of matrix metalloproteinases (MMPs), as there are specific NF-κB binding sites in the proximal regulatory region of MMP gene promoters[19]. These MMPs, a group of neutral proteolytic enzymes, degrade extracellular matrices and produce Zn2+ (ECM). Few direct effects of MMPs in periodontal tissue destruction and degradation are, destruction of connective tissue, loss of attachment and degradation of collagen, in which collagen fragments can stimulate or attract osteoclasts to cause alveolar bone absorption. According to Crudden et al[20,21], with the increasing degree of gingival inflammation, MMP activity and expression was also increased.

The expression of the IL18 protein is regulated by the IL18 promoter gene. Polymorphisms in anti-inflammatory or anti-inflammatory cytokine genes are associated with periodontal disease in different studies [22,23]. Several polymorphisms in the IL18 promoter region have been identified[24]. Two single nucleotide polymorphisms (SNPs) at positions –607 and –137 have been extensively studied in association with multifactorial diseases such as asthma, systemic lupus erythematosus, cardiovascular disease, and diabetes. Each of these two SNPs may be located in a transcription factor-binding element, which affects transcription of the promoter gene IL18.[24,25]. To our knowledge, only four studies have examined the association between IL18 polymorphisms at positions –607 and –137 and periodontal disease; these were performed in adult Italian >50 years[26], German subjects with mean ages 52.9[27] and 34.0[28] years. In the past, our team has had extensive experience carrying out various research projects in a variety of fields [29–45]. Now the growing trend in this field has motivated us to continue this project. However, a meta-analysis focusing on just three findings from Germany showed that the IL18 polymorphism was linked to a higher incidence of periodontal disease [23,46]. At present, most of the previous studies done for association between IL-18 and periodontitis were from European populations and Chinese populations and were done using older classification of periodontitis. Very few studies were done in the Indian population where they compared the levels of the cytokines IL-18 in GCF gingival tissue extracts from individuals with healthy gingiva and periodontitis [47–49] but using the older classification.

Hence, further research is needed with newer classification of periodontitis-AAP (2018) in order to understand and confirm if IL18 polymorphism affects periodontal disease in the Indian population. Thus, the aim of the present study was to analyse the association of IL-18 gene single nucleotide polymorphism (SNP) rs1946518 with periodontitis.

MATERIALS AND METHODS

A total of 100 individuals who reported to the Department of Periodontics were included in this cross sectional study.The sample size was calculated based on the previous study by Kaarthikeyan et al [50] based on which sample size of 100 was derived keeping the power of the study as 80%. The subjects were divided into a Periodontitis (stage II and III and grade B) group (n=50) and a control group (n=50) based on the clinical examination of probing pocket depth, clinical attachment loss, and bleeding on probing. The periodontitis group contained 50 patients (male-26; female-24) with a mean age of 39.02±8.22. The Periodontitis patients were recruited based on the criteria of the American Association of Periodontology (AAP)-2018 [51]. The control group contained 50 periodontally healthy and localised chronic gingivitis subjects (male-26; female-24) with mean age of 41.34±7.49.

Inclusion Criteria

Control group: Patients who are clinically healthy gingiva on an intact periodontium (AAP 2018 classification) (Bleeding on probing is <10%, pocket probing depth <3mm, no probing attachment loss, no radiological bone loss)

Test group: Patients who are systemically healthy, stage II and III (based on severity, complexity, extension and distribution) and grade B (moderate rate of progression)

Exclusion Criteria

Smokers, pregnant or lactating mothers, immunocompromised individuals, subjects who underwent periodontal therapy within the 6 months were excluded from this study.

The ethical clearance was obtained from the Institutional Review Board and written informed consent was obtained from all the patients who participated in the study.

Sample Collection

A volume of 2 ml of venous blood was obtained from the anterior pit and dispersed in a sterile tube containing a pinch of ethylenediaminetetraacetic acid (EDTA). It has been thoroughly mixed to prevent blood clots from forming. DNA isolation was performed according to the modified method of Miller et al. Protocol 1998[52].

Polymerase chain reaction and restriction endonuclease digestion

The polymorphism of the IL18 receptor gene rs1946518 was assessed by PCR amplification and digestion. The following primers, forward primer: 5'CCCTCTCCCCAAGCTTACTT3' and reverse primer: 5'TTCAGTGGAACAGGAGTCCA3' were used to amplify DNA covering the polymorphic MseI site of the IL18 receptor gene. DNA amplification was performed at a volume of 20 µl using 10 µg of genomic DNA, 5 µmol/µl forward and reverse primers with the PCR master mix (Takara, Japan). The cycling conditions were: initial denaturation at 94°C for 5 min, denaturation at 94°C for 35 s, annealing at 60°C for 35 s, stretching at 72°C for 35 s and elongation finally at 72°C for 5 min 5 µl PCR product was monitored on 2% agarose gel [Figure 1). 15 µl of PCR product was digested using the restriction enzyme SspI provided by New England Biolabs, UK. Digestion was performed at 37°C for 2 h. The digested product was visualized on a 2.5% agarose gel and the results recorded [Figure 2).

Statistical analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences Version 23.0 for Windows (SPSS Inc., Chicago, IL). The distribution of genotypes and allele frequencies in the chronic periodontitis and control groups were compared using the Chi-square test. 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 determined at P<0.05.

RESULTS

The clinical characteristics of the subjects in Periodontitis and control groups are shown in Table 1. The genotype and allele frequencies of the group are shown in Tables 2 and 3. The genotype frequency and distributions of IL-18 receptor MseI polymorphism did not differ significantly at χ2df(P=.818). Our study results showed that homozygous and heterozygous mutant genotypes had no significant difference (GG vs TG+TT) between the periodontitis patients and control group with a P-value of 0.8316. The detected frequency of TG (20% vs 22%) and TT (14% vs 10%) genotype showed significant differences between control and test group. There was no significant difference in G allele (76% vs 79%) and T allele (24% vs 21%) between the test and control group. From Hardy weinberg equilibrium, as χ2df (P=0.818) < 3.841, we can conclude that genotype frequencies in the population are not significantly different (Table 2).

DISCUSSION

To our knowledge, this is the first study in the South Indian population to examine the association between IL18 polymorphisms and periodontal disease. Our institution is passionate about high quality evidence based research and has excelled in various fields [53-63] The genetic polymorphism influences susceptibility of periodontitis and various gene polymorphisms are shown to play a role in periodontitis [64,65,66-68].IL-18 is a multipotent cytokine that has received a lot of attention in periodontitis because of its critical role in mediating the immune response of the host defence system. The present study is the first to study the role of IL- 18 gene polymorphisms in susceptibility to chronic periodontitis in the South Indian population.

The results of our study showed that there was no significant difference between homozygous and heterozygous genotypes (GG vs TG TT) between periodontitis patients and control group with a P value of 0.8316. The detection frequencies of the TG (20% vs 22%) and TT (14% vs 10%) genotypes showed no significant difference between the control and experimental groups. There were no significant differences for the G allele (76% vs 79%) and the T allele (24% vs 21%) between the experimental and control groups. We also confirmed that our genotype frequencies were not significantly different from those of the population.

The literature shows studies both in accordance as well as contradicting the hypothesis that IL-18 variants, alone or in combination, could have a correlation with periodontitis. In particular, Folwaczny[27] demonstrated that the distribution of haplotype combination for the IL-18 polymorphisms −607 and −137, showed no significant differences between the healthy control group and the periodontal patients. The population was only Caucasians. A study done in 2017 among South Japanese pregnant women with age groups between 18-45 years[69] had no significant association between polymorphism of rs187238 and periodontitis. In their study only pregnant women were involved and the periodontal examination for inclusion was carried out by a dental hygienist without any special training so the actual consistency of periodontitis might contradict the results. In the German population of age <40 years with Aggressive periodontitis (Ap)[28] also, the IL-18 gene mutations had no significant effect in periodontitis. In 2012, IL-18 −607 C/A and −137 G/C gene promoter polymorphisms were not associated with Periodontitis in the Italian population[26]. This study has detected a moderate association of C/A at position −607 (ExpB=2.099) with the healthy status compared to aggressive periodontitis but no association for chronic periodontitis was found. On the contrary, Chao Shan et al[70] also has reported results stating that there was a significant association between the IL-18 137 G > C and risk for periodontitis in the Chinese population, however the sample size was less. These literature evidence suggest that there is ethnic variability in the association between IL-18 polymorphism and periodontitis. Moreover, the disease classification might have an influence on the results. Many studies on aggressive periodontitis did not show any association whereas studies on chronic periodontitis had shown some association. In the present study, we have used the newer classification wherein there is not demarcation of chronic and aggressive periodontitis. When interpreting these results, this factor also should be considered.

IL-18 gene polymorphism is also associated with various systemic diseases. A meta-analysis on IL-18 polymorphisms showed that IL-18 −607 C/A and −1297 C/T polymorphism were associated with the development of Systemic lupus erythematosus(SLE) in Europeans, and the IL-18 −137 G/C polymorphism were associated with SLE in Asians[71]. Htoon et al[72] in their study reported that there was significant association between IL18 gene polymorphism and Behcet's syndrome, whereas they reported there was no significant association between IL 18 gene polymorphism and systemic lupus erythematosus. In the Turkish population, Palomino-Morales et al[73] have reported a significant association between allele “A" in rs1946518 and giant cell arteritis. In addition, the association between rs187238 and Henoch-Schönlein purpura has also been reported[74]. This suggests that IL18 might be a common susceptibility locus for at least 3 vasculitic diseases.

At present, most of the previous studies done for association between IL-18 and periodontitis are from different populations

as well as small sample size and were done using older classification of periodontitis. The present study was limited to genetic polymorphism in periodontitis (stage II and III and grade B) patients excluding all the confounding factors of periodontitis and was done based on the latest criteria of American Association of Periodontology (AAP)-2018; further studies using the newer classification with larger sample size in various ethnic groups are needed to confirm the influence of IL-18 gene polymorphism in periodontitis.

CONCLUSION

The present study denotes that IL18 gene polymorphism is not associated with periodontitis (stage II and III and grade B).

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Consent for publication

The patients have given valid and informed consent for publication.

Declaration of patient consent

The authors certify that we have obtained all appropriate patient consent forms. In the form, patients consented to have their images and other clinical information reported in the diary. Patients understand that names and initials will not be published and will endeavor to remain anonymous, but anonymity cannot be guaranteed.

Conflict of interest

No Conflict of interest

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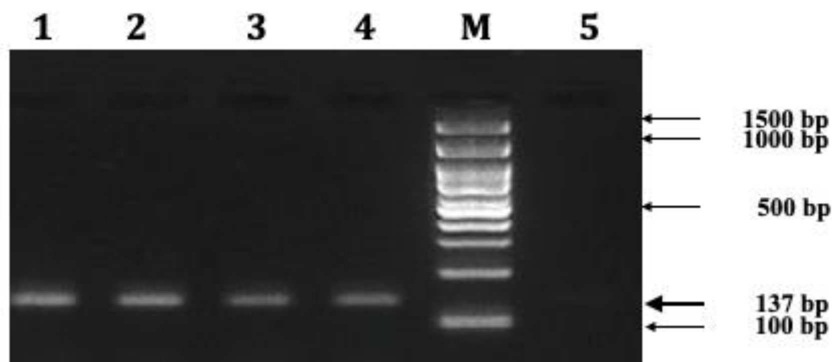

Figure 1: Agarose gel electrophoretogram of T>G polymorphism of IL-18 (rs1946518) showing 137 bp amplicon in lanes 1-4 (Lanes [M]: 100 bp DNA ladder), Lane 5: Negative control.

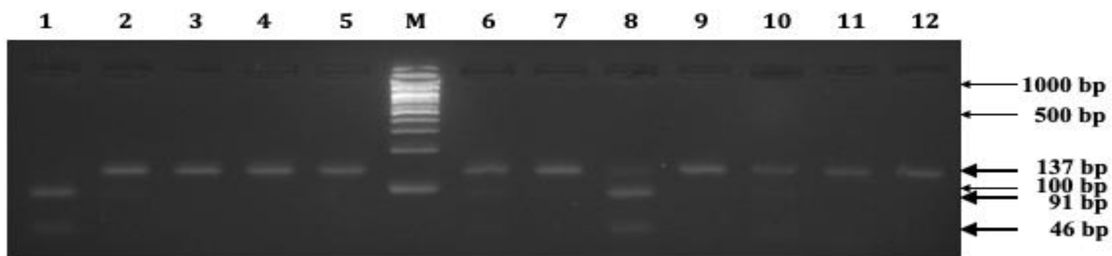

Figure 2: MseI digestion of PCR amplified product (Lanes 1, 8 (partially digested) -TT homozygous wild type, lanes 2-5, 7,9,11,12 - GG- Homozygous variant, lane 6 and 10 - GT - Heterozygous, Lane M-100 bp DNA ladder).

Table 1: Demographic data of periodontitis(stage II and III and grade B)and periodontally healthy control groups

Clinical Characters	Periodontitis group			Control group		
Number of subjects	Male	Female	Total	Male	Female	Total
	26	24	50	26	24	50
Mean age	39.02±8.22			41.34±7.488		
Clinical attachment loss	6.13±1.29			-		
Probing Pocket depth	5.48±1.15			1.60±0.57		
Gingival index	1.74±0.22			0.76±0.16		

Table 2: Genotype frequencies of IL-18 (rs1946518) gene T>G polymorphism among the cases and controls

Groups	**GG**	**TG**	**TT**	**G**	**T**	**HWE (p value)***
Case (N=50)	33	10	7	0.76	0.24	0.0014**
Control (N=50)	34	11	5	0.79	0.21	0.0171**

*For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom. The genotype frequency of cases and controls do not differ significantly x 2df (**P = 0.818**).

Table 3: Overall genotype distribution of the IL-18 (rs1946518) gene polymorphism in cases and controls based on models.

Dominant				
Genotypes	**Case**	**Control**	**Unadjusted OR [95% CI]**	**P value**
GG	33	34	0.9135 [0.3968 - 2.1032]	0.8316
TG + TT	17	16		
Recessive				
TG + GG	43	45	0.6825 [0.2012 - 2.3151]	0.5399
TT	7	5		
Allele				
G	76	79	0.8418 [0.4329 - 1.6368]	0.6117
T	24	21		