Association of Interleukin-1β +3954 (Rs1143634) Gene Polymorphism in Periodontal Healthy and Chronic Periodontitis Patients

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Abstract:

Background: Polymorphisms of various cytokine genes are known to influence the pathogenesis and severity of several inflammatory conditions. The association of these polymorphisms with chronic periodontitis (CP) susceptibility has been found to be diverse amongst different ethnic populations. This study was aimed to determine the distribution of IL-1β+3954 (rs1143634) gene polymorphism in healthy controls (HC) and CP and to explore their association with periodontal status.

Methodology: The above polymorphism was analysed amongst HC (n=56) and CP patients (n=79). Genotyping was performed using PCR-RFLP assay. Mann-Whitney U test and Kruskal Wallis ANOVA test were applied to find out the differences in genotypes of SNPs with clinical parameters and chronic periodontitis.

Results: No significant correlation was found between IL-1β+3954 (rs1143634) gene polymorphism and both groups though the frequencies of C/C genotype and allele T were higher in CP.

Conclusion: Lack of association of IL-1β+3954 (rs1143634) gene polymorphism was observed with chronic periodontitis in the population of Northern Karnataka.

Keywords: Interleukin-1 beta, polymorphism, chronic periodontitis, gene, allele, genetics

I. Introduction

Periodontal disease is known to be a complex disease having multifactorial etiology. The interaction between the host and bacteria is important for periodontal disease pathogenesis. This involves cytokine mediated complex interactions between cells and the extracellular matrix. Complex diseases are typically polygenic, inflammatory and immune responses in periodontitis can be influenced by genetic factors. The evidence of the genetic basis for periodontitis has been supported by familial aggregation, twin, segregation, linkage and association studies. Reports on twin studies show the heritability of periodontal disease. Also chronic periodontitis having familial aggregations was analysed with familial study designs.

Role of genes and the polymorphisms in host responses leading to disease progression has been reported widely in the literature. Genetic polymorphisms may be a risk factor for the disease susceptibility and may be protective for a disease. Genetic polymorphism in several aspects of immunity have been investigated as the host immune response has a major role in the pathogenesis of periodontitis. There are many candidate genes investigated in relation to chronic periodontitis. Cytokines have a significant role in inflammation and immunity. IL-1 is a key cytokine in many chronic diseases such as rheumatoid arthritis, Alzheimer’s disease and periodontitis. IL-1β is mainly produced by activated macrophages and fibroblasts that release this extracellular protein. The gene regulating the production of IL-1β is located on the chromosome 2q14.2. IL-1β gene has three polymorphisms based on transitions between C and T at positions +3954/3953 (C>T, rs1143634), -511 (C>T, rs16944), and -31 (T>C, rs1143627) base pairs from the transcriptional site. The host mediated interactions determining the disease phenotype can be understood with cytokine gene

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polymorphisms studies. Several SNPs have been evaluated to determine the susceptibility of chronic periodontitis in various populations of the world with inconsistent results. In this study, we have analysed the association between SNP in IL1β (+3954) polymorphism in chronic periodontitis susceptibility in a hospital-based sample population from northern Karnataka, India.

II. Material & Methods

This case control study had 135 patients visiting the outpatient Department of Periodontics and Oral Implantology, Shri Dharmasthala Manjunatheshwara College of Dental Sciences and Hospital (SDMCDSH), Dharwad, India, between March 2016 and April 2017. An ethical clearance was obtained from the institutional ethical committee and informed written consent was obtained from all the patients before their participation in the study. The study population was comprised of 63 males and 72 females. The inclusion criteria were patients of 18 to 60 years old divided into two groups. Group 1 (Healthy controls) was comprised of 56 patients with no history of previous periodontal disease, no signs of gingival inflammation, absence of clinical attachment loss and no sites with probing depth > 3mm. Group 2 (Chronic Periodontitis) was comprised of 79 patients diagnosed with chronic periodontitis based on presence of inflammation, loss of clinical attachment, and loss of adjacent supporting bone as evidenced by radiographic examination. They had >30% of sites exhibiting a clinical attachment loss of ≥ 5mm and at least three teeth exhibiting sites of clinical attachment loss in at least two different quadrants. The exclusion criteria were tobacco smokers/chewers, immunosuppressed subjects, any patient who has undergone periodontal therapy in the last 3 months, any history of systemic disease, history of antibiotic intake in the past 3 months, pregnant and lactating women. The clinical parameters assessed were plaque index (PI)\(^1^2\), gingival index (GI)\(^1^2\), probing pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP).

Four mL of peripheral blood mononuclear cell (PBMC) samples was collected from all patients in EDTA coated vacutainers. The collection tubes were coded to maintain confidentiality and transported to the laboratory in ice-cold condition (4°C). DNA was isolated using standard isolation kit (DNeasy Blood and Tissue Kit, Cat. No. 69506, QIAGEN, USA). DNA quantification and purity was assayed using UV spectrophotometer at 260nm and 280nm.

<table>
<thead>
<tr>
<th>Table 1: Primer sequences and reaction conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primer Name</strong></td>
</tr>
<tr>
<td>IL-1β, +3954C/T (rs1143634) Forward primer</td>
</tr>
<tr>
<td>IL-1β, +3954C/T (rs1143634) Reverse primer</td>
</tr>
</tbody>
</table>

The 25μl PCR reaction mixture was prepared that had 0.2 μM forward and reverse primer each; 200 M of dNTPs (NEB #NO447L); 1X Taqbuffer, 1unit Taq enzyme (NEB #MO273L); about 1000ng of DNA made up to volume with nuclease free water. Initial denaturation for 30 seconds at 95°C followed by 35cycles of denaturation at 95°C for 15 seconds and extension at 68°C for 1 minute. After the PCR amplification, the amplicons were run through 4% agarose gel electrophoresis and the DNA bands were observed in gel documentation. All PCR products (10μl) were digested with 10units of restriction endonuclease enzyme. Restriction enzyme digestion was carried out by incubating at 37°C for 15 minutes (Table 1).

The restriction enzyme digestion products were subjected to agarose gel electrophoresis. 4% agarose was prepared in 1X TAE buffer (300ml). To this 30μl of ethidium bromide was added. 5μl of restriction enzyme digest product was mixed with 3μl of loading dye bromophenolblue. The electrophoresis was conducted at a constant voltage of 100V for 60 minutes. The observed bands were analysed under the gel documentation chamber (Fig 1).

**Statistical Analysis:** The mean and standard deviation were calculated for all the continuous variables and frequency and percentage were calculated for all the categorical variables. The association of allele and genotype frequencies for the SNP with chronic periodontitis was assessed by Chi-square test. The odds ratio (OR) was determined to estimate the risk of developing severe periodontitis. The statistical analysis was carried out by
The comparison of all these parameters in cases and controls was statistically significant. No significant association between genotypes of IL-1β +3954 with clinical parameters was found (Table 3). Most of the cases (86%) had the severe type of disease (localized/ generalized) having ≥5 mm clinical attachment loss. The remaining (14%) had the moderate type of disease with 3–4 mm of clinical attachment loss; none had the mild type of disease. Also, no statistical significance was found when compared with severity of chronic periodontitis (Table 4).

### Table 2: Clinical characteristics and parameters of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chronic periodontitis</th>
<th>Control</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD) (yrs)</td>
<td>40.42±8.72</td>
<td>32.64±5.94</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>35/44</td>
<td>28/28</td>
<td>0.531</td>
</tr>
<tr>
<td>PI (mean ± SD)</td>
<td>3.15±11.06</td>
<td>0.66±0.45</td>
<td>0.0001*</td>
</tr>
<tr>
<td>GI (mean ± SD)</td>
<td>3.16±11.05</td>
<td>0.11±0.24</td>
<td>0.0001*</td>
</tr>
<tr>
<td>PPD (mean ± SD) (mm)</td>
<td>4.31±1.18</td>
<td>2.02±0.13</td>
<td>0.0001*</td>
</tr>
<tr>
<td>CAL (mean ± SD) (mm)</td>
<td>5.92±1.46</td>
<td>1.91±0.29</td>
<td>0.0001*</td>
</tr>
<tr>
<td>BOP (mean ± SD) (mm)</td>
<td>1.00±0.00</td>
<td>2.00±0.00</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

No significant association of the alleles with the healthy controls and chronic periodontitis patients. The odds ratios for the genotypes in cases and controls were OR:0.76 (95% CI:0.24–2.41) for IL-1β genotypes and for the alleles C and T were OR:0.77 (95% CI:0.25–2.47) (Table 5).

### Table 4: Association between severity of chronic periodontitis and genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Severe CP</th>
<th>Moderate CP</th>
<th>Total</th>
<th>Chi-square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>25</td>
<td>35.71</td>
<td>45</td>
<td>64.29</td>
<td>88.61</td>
</tr>
<tr>
<td>C/T</td>
<td>4</td>
<td>66.67</td>
<td>2</td>
<td>33.33</td>
<td>6</td>
</tr>
<tr>
<td>T/T</td>
<td>1</td>
<td>33.33</td>
<td>2</td>
<td>66.67</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 5: Genotype and allele frequencies of cytokines in cases and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases</th>
<th>%</th>
<th>Controls</th>
<th>%</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β +3954</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>70</td>
<td>88.61</td>
<td>51</td>
<td>91.07</td>
<td>0.76</td>
<td>0.24</td>
<td>2.41</td>
</tr>
<tr>
<td>C/T</td>
<td>6</td>
<td>7.59</td>
<td>3</td>
<td>5.36</td>
<td>0.56</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>3</td>
<td>3.8</td>
<td>2</td>
<td>3.57</td>
<td></td>
<td>0.945</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>75</td>
<td>94.94</td>
<td>54</td>
<td>96.43</td>
<td>0.77</td>
<td>0.25</td>
<td>2.47</td>
</tr>
<tr>
<td>T</td>
<td>9</td>
<td>8.93</td>
<td></td>
<td></td>
<td></td>
<td>0.6436</td>
<td></td>
</tr>
</tbody>
</table>

Differences were significant (p<0.05)

Agarose gel electrophoresis images representative of genotyping experiment results by PCR-RFLP are depicted in figure-1.
Association of Interleukin-1β +3954 (Rs1143634) Gene Polymorphism in Periodontal Healthy and

Fig 1: Agarose Gel (4%) Electrophoresis gel image of interleukin-1β +3954 (rs1143634) gene polymorphism

(Line 1: 100bp DNA Ladder (marker), Line 2: PCR product of 250bp of CP, Line 3: PCR product digested with Taq 1 enzyme producing undigested fragment of 250bp (T/T genotype of CP), Line 4: PCR product digested with Taq 1 enzyme producing digested fragments of 250bp; 136bp and 114bp (C/T genotype of CP), Line 5: PCR product digested with Taq 1 enzyme producing digested fragments of 136bp and 114bp (C/C genotype of CP), Line 6: negative control).

IV. Discussion

Periodontal disease is a complex disease having the involvement of disease-modifying genes and their effect is modified by environmental factors.20 These factors are bacterial plaque, smoking, coffee and alcohol consumption, stress and systemic factors which may aggravate the pathology associated with the disease.21 The initiation and progression of chronic inflammatory diseases is regulated by cytokines. These are involved in the pathogenesis and clinical variability of chronic periodontitis. Their expression can be affected by the presence of polymorphisms in cytokine genes thus they play an important role in resistance and susceptibility to periodontal disease.22 Scientists stipulated that about 50% of the clinical differences of periodontal disease are evolved from gene polymorphisms.4 IL-1β +3954 polymorphism can be associated with an increase in IL-1β production. IL-1β has an important role in the pathogenesis of periodontal disease. Pociot et al. found that monocytes from these patients produced more IL-1β than patients without IL-1β +3954 polymorphism.23 Also, neutrophils from patients with periodontitis carrying IL-1 β+3954 had increased IL-1β levels compared to patients without this polymorphism but these levels were not significant.23 While allele 2 of the IL-1β polymorphism associated with decreased IL-1β secretion was found by Santtila et al.24

The present analysis revealed that the C/C genotype of IL-1β +3954 polymorphism was more prevalent than C/T and T/T genotypes in CP than HC while the C allele was slightly more frequent among HC when compared with CP while T allele was more frequent in CP. But this result did not reach the statistical significance. Allele T has been shown to be associated with severity of the periodontal disease.20 Similar findings were reported by Shete et al.25 where the frequency of the CC genotype but in contrast Callele was more frequent with chronic periodontitis.

A negative association was also documented in Northern Europe, Poland, Jordan and South African populations.25,26 The CT genotype was found to be a significant risk factor for the development of the disease in North Indian population and a positive association of this variant was observed in studies from Brazil and South Indian states.30,31,32,33,34 A meta-analysis supporting a positive association documenting the CT genotype of chronic periodontitis patients as a significant risk factor in Caucasians was observed but not in Asians35 and the another included 53 studies with 4178 cases and 4590 controls reporting its strong association with disease susceptibility.36 Lack of significant association of IL-1β +3954 polymorphism with the clinical parameters such as pocket probing depth, clinical attachment level, plaque and gingival indices also with the severity of chronic periodontitis was observed.

There is a large inconsistency of genetic polymorphisms in different studies suggesting the presence of several genetic profiles for the forms of periodontitis in different populations or the diversity may be due to the detection methods and also the inter-individual variation in cytokine production. The protein product of the

DOI: 10.9790/0853-1708035863 www.iosrjournals.org 61 | Page
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Abstract

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Key Points

- Genetic polymorphisms in periodontal diseases
- Association with disease status
- Role of cytokines
- Future strategies

References

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