INTRODUCTION
Periodontitis is a chronic inflammatory disease which has a multifactorial etiology. Although the presence of Gram-negative anaerobic bacteria is essential for the initiation of periodontal destruction, many environmental and genetic factors could result in the progression of the disease. The genetic influence plays a key role in determining the host susceptibility to periodontal destruction.[1,2] Many studies have attempted to identify the genetic factors that may be related to enhanced susceptibility to periodontal disease.[3-8]

Periodontal health requires balance between tissue destruction enzyme such as matrix metalloproteinases (MMPs) and their inhibitors. MMP is a large family of zinc-dependent extracellular protease which is responsible for the tissue remodeling and degradation of the extracellular matrix, including collagens, elastins, gelatin, matrix glycoproteins, and proteoglycans.[9] Most of the member of MMPs family are secreted as its inactive preform.[10] The proteolytic activities of MMPs are precisely regulated by the involvement of key factors and MMPs getting activated from their precursors.[11] Studies suggest that MMPs comprise the important pathway toward the tissue destruction and remodeling associated with periodontal tissue.[12] MMP8, also called neutrophil collagenase, is one of the major collagenolytic enzymes that can initiate the digestion of Type I collagen. It is released from leukocytes during chemotactic stimulation in vitro and in response to inflammatory condition in vivo.[13-15] In oral fluids, gingival crevicular fluid and saliva, MMP8 levels are associated with the initiation and progression of periodontitis.[16-18]

Recent studies suggest that in addition to tissue destructive properties, MMP8 can also exert anti-inflammatory effects in the host defense by processing anti-inflammatory cytokines and chemokines.[14,19]

Several common single-nucleotide polymorphisms (SNPs) have been identified in the MMP8 gene and located on chromosome 11.[20] Three of them at position -799C/T (rs11225395), -381A/G (rs1320632), and +17C/G (rs2155052) from the major transcription start site (accession number AF059679) have been
described to have putative function significance. However, extent to which these MMP8 variants might affect serum MMP8 concentration in vivo is not known. To date, there is minimal study that has analyzed allele, genotype, or haplotype frequencies of MMP8 polymorphisms in patients with periodontitis.

We hypothesized that MMP8 polymorphisms might modify the relative risk for the development of chronic periodontitis (CP). On the basis of these findings, we evaluated SNP [-799C/T (rs11225395)] in MMP8 gene in groups of non-periodontitis controls and periodontitis participants.

MATERIALS AND METHODS

This study employed a cross-sectional design involving individuals from the South Indian population. A total of 100 individuals who reported to the Department of Periodontics, Tamil Nadu, were included in this study. The participants 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 (male – 26 and female – 24) with the mean age of 39.02±8.22. The CP patients were recruited based on the criteria of American Academy of Periodontology-1999. The control group contained 50 periodontally healthy participants (male – 26 and female – 24) with mean age of 41.34±7.49.

A detailed history of dental treatment, family history of periodontal diseases, smoking habits, as well as general health concerns were obtained from the participants. Except for the presence of periodontitis, the patients included in this study were systemically healthy. Smokers, pregnant or lactating mothers, immunocompromised individuals, and participants who underwent periodontal therapy within the past 6 months were excluded from this study. The study was approved by the Institutional Ethical Committee (SRB/MDS/PERO/18-19/0004).

Sample Collection and Deoxyribonucleic Acid Extraction

A volume of 2 ml of venous blood was collected from antecubital fossa and dispersed into a sterile tube containing a pinch of ethylene diamine tetraacetic acid. It was mixed thoroughly to avoid clot formation. DNA isolation was performed according to the modified Miller et al. 1998 protocol.

Polymerase Chain Reaction and Restriction Endonuclease Digestion

MMP8 (-799C/T) gene (SfcI) polymorphisms were assessed by PCR amplification and restriction digestion. The following primers, forward primer: 5'-CTGTGTGAAGCCCTAGAGCCTGGCTCC-3' and reverse primer: 5'-GATCTTCTTTCTAACTCTACCC-3', were used for amplification of DNA spanning the SfcI polymorphic site, of the MMP8 gene. The amplification of DNA was performed in 20µl volumes using 10ng of genomic DNA and 5 pmol/µl each of forward and reverse primers along with PCR master mix (Takara, Japan). The cycling conditions were as follows: Initial denaturation at 94°C for 5 min, denaturation at 94°C for 35 s, annealing at 60°C for 35 s, extension at 72°C for 35 s, and a final extension at 72°C for 5 min. 5 µl of PCR product was checked on a 1% agarose gel. 15 µl of PCR product was digested using SfcI restriction enzyme procured from New England Biolabs, England. Digestion was carried out at 37°C for 2 h. The digested product was visualized on a 2% agarose gel, and the results were documented.

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 CP and control groups was 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 participants in CP and control groups are shown in Table 1. The genotype and allele frequencies of the groups are shown in Table 2. The genotype frequency of MMP8 (-799C/T) SfcI polymorphism does not differ significantly (P = 0.97531). Our study results showed that the prevalence of homozygous and heterozygous mutant genotype had no significance.
(CC vs. CT+TT) difference between CP and healthy control group. The detected frequency of CC (32% vs. 30%), CT (44% vs. 46%), and TT (24% vs. 24%) genotypes had no significant difference between CP group and healthy controls. There was no significant difference in C allele (54 vs. 53) and T allele (46 vs. 47) between CP and healthy control group [Table 3].

**DISCUSSION**

Genetic polymorphisms, like SNPs, may influence disease in multiple complex ways acting with other genetic variants and environmental factors to influence disease susceptibility and progression. Many studies have revealed that SNPs may be associated with susceptibility to periodontitis.[1]

MMP8 is a promising biomarker candidate for periodontitis. It is synthesized by differentiating granulocytes in the bone marrow and stored in specific granules of circulating neutrophils.[23] Thus, synthesis and activation of MMP-8 are important steps in the pathological extracellular matrix destruction associated with the periodontal disease.[24] Inflammatory cells such as neutrophils and macrophages produce MMPs, with neutrophils being the major cause source of gelatinase and collagenase in inflammatory diseases such as periodontitis. Epithelial cells can also produce elevated levels of these enzymes, which may facilitate apical migration and lateral extension of the junctional epithelium and the subsequent loss of connective tissue attachment. Inflammatory cells, particularly neutrophils, play a particularly important role in the MMP-8-mediated periodontal destructive lesion. These concepts gave the insight to design the current study to analyze the association of MMP8 gene polymorphism and CP.

Our study results showed that the genotype frequency of MMP8 SfcI polymorphism did not differ significantly. The prevalence of homozygous and heterozygous mutant genotype had no significant difference between CP and healthy control group. The detected frequency of CT and TT genotypes had no significant difference between CP group and healthy controls. There was no significant difference in C allele and T allele between CP and healthy control group.

Izakovicova Holla et al.[10] reported that there was no significant difference in the allele and genotype frequencies of the MMP8-799C/T and +17C/G polymorphisms between patient with CP and control among Czech population which was in association to our present study. Chou et al.[25] reported that the frequency of genotypes in chronic and aggressive periodontitis patients was similar but was significantly different from those in healthy controls among Taiwanese population. They suggested that no smoking Taiwanese with MMP8-799 T allele was associated with the risks of both chronic and aggressive periodontitis. The difference in the results from the present study could be because of different ethnicity and due to small sample size in the present study.

Since the present study was subjected to one particular ethnicity, the future studies need to evolve such that multicenter studies are conducted to have better understanding of MMP8 gene polymorphism among various populations. The strength of the present study is that all the confounding factors such as smoking and systemic disorders were excluded during the recruitment of the sample. Further studies are required to explore the interaction of gene with microbial and environmental factors in the etiopathogenesis of periodontitis.

### Table 2: Genotype frequencies of MMP8 (-799C/T) (rs11225395) polymorphism among the cases and controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Genotype frequency (%)</th>
<th>Allele Frequency</th>
<th>HWE P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>16 (32)</td>
<td>C=0.54</td>
<td>0.418</td>
</tr>
<tr>
<td>CT</td>
<td>22 (44)</td>
<td>T=0.46</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>12 (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>15 (30)</td>
<td>C=0.53</td>
<td>0.587</td>
</tr>
<tr>
<td>CT</td>
<td>23 (46)</td>
<td>T=0.47</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>12 (24)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*For departure from Hardy–Weinberg equilibrium (HWE), Chi square with one degree of freedom.

The genotype frequency of cases and controls does not differ significantly \(X^2_{0.05}(1)=0.97531\).

### Table 3: Genotype distribution of the MMP8 (-799C/T)(rs11225395) polymorphism among the cases and controls

<table>
<thead>
<tr>
<th>Models</th>
<th>Cases N=50</th>
<th>Controls N=50</th>
<th>Unadjusted OR [95% CI]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dominant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>16</td>
<td>15</td>
<td>1.0980 [0.4703 to 2.5637]</td>
<td>0.82</td>
</tr>
<tr>
<td>CT+TT</td>
<td>34</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Recessive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>12</td>
<td>12</td>
<td>1.0000 [0.3994 to 2.5039]</td>
<td>1.00</td>
</tr>
<tr>
<td>CT+CC</td>
<td>38</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Additive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>54</td>
<td>53</td>
<td>1.0410 [0.5972 to 1.8148]</td>
<td>0.8873</td>
</tr>
<tr>
<td>T</td>
<td>46</td>
<td>47</td>
<td></td>
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</tbody>
</table>
CONCLUSION

Thus, the present study concludes that the MMP8 (-799C/T)(rs11225395) polymorphism had no significant association in CP. Further studies involving other ethnic populations and the functional mechanisms of SNP are needed to confirm the findings in this article.

REFERENCES


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