Distribution of Matrix Metalloproteinase–9 (Mmp-9) –1562 C/T Gene Polymorphism in Indonesian Males with Periodontitis

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Abstract
The aim of this research was to compare the distribution of the MMP-9 -1562 C/T (rs3918242) polymorphism in Indonesian males with and without periodontitis. This descriptive study used 100 stored biological samples. The variation in the MMP-9 (-1562 C/T) polymorphism was investigated by the polymerase chain reaction (PCR) – restriction fragment length (RFLP) method with SphI restriction enzyme digestion and electrophoresis of the resulting fragments. A significant difference was found for MMP-9 -1562 C/T genotypes and alleles between subjects with periodontitis and healthy controls (p < 0.05). The T allele was relatively uncommon, as it appeared in 2% of healthy controls, with no TT genotype observed in subjects with or without periodontitis. Nevertheless, the T allele and CT genotype appeared to be significantly associated with the risk of periodontitis. The results suggested that the T allele of the MMP-9 -1562 C/T polymorphism is significantly associated with periodontitis.

Keywords: Gene polymorphism, periodontitis, MMP-9, Indonesia.

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Introduction
Periodontal diseases has a high (up to 60%) prevalence in the Indonesian population, and the prevalence of periodontitis (up to 53%) in South East Asia ranks third in the world.1,2 Periodontitis is a multifactorial disease caused by the interaction of specific oral microorganisms and host factors and resulting in a progressive destruction of the periodontal ligament and alveolar bone.3 Specific microorganisms initiate periodontitis, but genetic factors of the host also contribute to its progression.4 One suggestion is that genetic factors, including the polymorphism of the MMP-9 gene, are related to alveolar bone destruction in periodontitis.5 MMP9 is a gene of the matrix metalloproteinase (MMP) family that produces important enzymes for inflammatory mediation and the physiological degradation of the extracellular matrix during tissue remodeling.5

An imbalance between MMPs and their host inhibitors, the tissue inhibitors of metalloproteinase (TIMPs), could lead to periodontitis by initiating bone destruction. MMPs are produced and secreted by host cells, such as fibroblasts, keratinocytes, endothelial cells, osteoclasts, neutrophils, and macrophages, but they are also produced by several periodontal pathogens, such as Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis.3

One MMP that is predominant in periodontitis is MMP-9, also known as gelatinase. B. MMP-9 plays a key role in the degradation of collagenases type IV and the extracellular matrix.6 The target components of the MMP-9 gelatinase are fibronectin, elastin, collagenases type IV, V, VI, and X, and type I denatured collagen. These components are all involved in the inflammatory destructive process of periodontitis.7-11

MMP-9 secreted by neutrophils is very effective at degrading type I and type IV collagen,
which is the most abundant collagen type in the periodontal ligament.\textsuperscript{3,11}

MMP-9 also plays a role in the degradation of non-collagenous components in the extracellular matrix.\textsuperscript{11}

MMP-9 has been found in neoplastic disease and non-neoplastic disease. Increased MMP-9 levels have been found in the gingival crevicular fluid (GCF) and the gingival tissues of patients with periodontitis.\textsuperscript{5,6,11} The MMP-9 gene with polymorphism at -1562 C/T (rs3918242) is located at 20q11.2–13.1. The influence of this gene and its polymorphism in the etiology of periodontitis has been previously reported for Turkish, Chinese, and Brazilian populations based on PCR-RFLP methods.\textsuperscript{5,8,9,10}

No studies have been reported on the distribution of MMP-9 -1562 C/T (rs3918242) polymorphisms related to periodontitis in the Indonesian population. The aim of this study was to compare the distribution of the MMP-9 -1562 C/T (rs3918242) genetic polymorphism in Indonesian males with and without periodontitis.

Methods

Patients

In total, 50 Indonesian males with periodontitis and 50 healthy control subjects were included after obtaining written informed consent signed by each participant. This study was approved by the ethics committee of the Faculty of Dentistry, University of Indonesia.

Subject selection and DNA isolation

A total of 100 DNA samples were obtained from blood serum of patients with periodontitis (50 samples) and healthy controls (50 samples). All samples were stored at –20°C in the laboratory of Oral Biology, Faculty of Dentistry, Universitas Indonesia. DNA isolation was performed according to previous methods reported by Auerkari et al.\textsuperscript{12,13}

Polymerase chain reaction amplification (PCR)

The polymorphic site was amplified using two primers: forward (5’–TTC GTG ACG CAA AGC AGA–3’) and reverse (5’–AGC AGC CTC CCT CAC TCC T–3’). The polymerase chain reaction (PCR) was performed in a total volume 25 μL containing 12.5 μL KAPA taq Ready Mix PCR with dye (Kapa Biosystem, USA), 0.5 μL forward primer, 0.5 μL reverse primer, 0.3 μL DNA, and 11.2 μL ddH\textsubscript{2}O.

PCR was conducted with an initial denaturation at 95°C for 3 minutes. The amplification was then performed with 35 cycles of denaturation at 95°C for 1 minute, annealing at 60.7°C for 45 seconds, and extension at 72°C for 45 seconds. The final extension was at 72°C for 5 minutes, and a final hold at 4°C.

The PCR product was electrophoresed in a 1.5% agarose gel (Thermo Scientific, USA) at 50V/400mA for 45 min, and visualized using GelDoc to show the 560 bp amplification product.

Restriction fragment length polymorphism (RFLP)

For RFLP, the PCR product (10 μL) was digested with 0.5 U of SphI restriction enzyme (Gene Mark, Taiwan) in 2 μL 10 × GM-Buffer II (Gene Mark, Taiwan), and 5.9 μL ddH\textsubscript{2}O, and incubated overnight (16 h) at 37°C for enzyme activation. The enzyme was then inactivated in a thermo block at 65°C for 20 min. The RFLP product was electrophoresed in 2% agarose gel (Thermo Scientific, USA) at 50V/400mA for 45 min and visualized using GelDoc.

The RFLP products can, in principle, correspond to the genotypes CC (560 bp), CT (560, 300, and 260 bp), and TT (300 and 260 bp).

Statistical analysis

Chi-square and Fisher exact tests were used to compare the genotype and allele distributions in the groups with and without periodontitis, and to test for the compatibility of the results with the Hardy-Weinberg Equilibrium. Statistical significance was assumed for p < 0.05.

Results

The PCR product from DNA amplification of the MMP-9 in the promoter region -1560 was 560 bp. After using the cutting enzyme SphI for polymorphism status, the resulting variants of MMP-9-1562 C/T (rs3918242) were CC (wild-type homozygote) with a single band, and CT (heterozygote) with three bands. The TT (mutant homozygote) genotype with two bands was not observed (Table 1). The genotype distribution was consistent with the Hardy-Weinberg equilibrium for both periodontitis and control groups (p>0.05).
Distribution of Matrix Metalloproteinase-9

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Table 1. Genotype and allele distributions of MP-9 -1562 C/T polymorphism.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Periodontitis (n = 50)</th>
<th>Healthy controls (n = 50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>39 (78%)</td>
<td>48 (96%)</td>
<td>0.015</td>
</tr>
<tr>
<td>CT</td>
<td>11 (22%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>NI</td>
<td>NI</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Periodontitis (n = 50)</th>
<th>Healthy controls (n = 50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>89 (89%)</td>
<td>98 (98%)</td>
<td>0.018</td>
</tr>
<tr>
<td>T</td>
<td>11 (11%)</td>
<td>2 (2%)</td>
<td></td>
</tr>
</tbody>
</table>

NI = Not identified.

The wild-type homozygote (CC) genotype and allele C were most common, at a frequency of 78 and 89%, respectively, in the periodontitis group and 96 and 98%, respectively, in the control group. No TT genotype was observed, but the heterozygote (CT) genotype and T allele were significantly more frequent in the periodontitis group than in the control group. This may suggested that the T allele was significantly associated with an elevated risk of periodontitis.

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Declaration of Interest

The authors report no conflict of interest.

References