

+17 C/G POLYMORPHISM IN MATRIX METALLOPROTEINASE (MMP)-8 GENE AND ITS ASSOCIATION WITH PERIODONTITIS

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ABSTRACT

INTRODUCTION: Matrix metalloproteinase-8 (MMP-8), encoded by *MMP-8* gene (chromosomal location, 11q22.3), is a proteolytic enzyme that is involved in the pathogenesis of periodontitis. Polymorphism of the encoding *MMP-8* gene can affect the risk of the disease.

OBJECTIVES: This study aimed to evaluate the relationship between +17 C/G polymorphism in *MMP-8* and periodontitis in an Indonesian sample population.

MATERIAL AND METHODS: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the +17 C/G polymorphism in *MMP-8* was conducted in stored genomic DNA samples from 100 subjects with periodontitis and 100 healthy controls. Patients were examined in a previous study by a periodontics and diagnosed as periodontitis based on anamnesis and clinical examination (periodontal pocket depth \geq 4 mm and the presence of clinical attachment loss).

RESULTS: The frequencies of the genotypes or alleles in the periodontitis and control groups were not significantly different (genotype, $p = 0.283$; alleles, $p = 0.11$). Any remaining trend toward higher frequencies of the polymorphic genotypes or alleles in the periodontitis group was explained by the adjustment for age.

CONCLUSIONS: The results showed no significant association between the +17 C/G polymorphism in *MMP-8* and the risk of periodontitis in the Indonesian population. No change in the significance is expected even with a considerably larger sample.

KEY WORDS: MMP-8, periodontitis, gene polymorphism, +17 C/G polymorphism.

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INTRODUCTION

Periodontitis is a common inflammatory disease that results in a progressive destruction of the periodontal ligament, following in loss of alveolar bone and teeth during the final untreated stages. About half or more of the adult population in the world is affected by periodontitis, and the prevalence is increasing with age, unhealthy diet, smoking habits, and poor oral hy-

giene [1-3]. It is the most common (61%) disease affecting the general population in Indonesia [4].

Periodontitis is a multifactorial disease, thought to be initiated via gingival and periodontal inflammation, which is promoted by anaerobic bacterial pathogens such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium nucleatum*, and/or *Aggregatibacter actinomycetem comitans* [5]. The pres-

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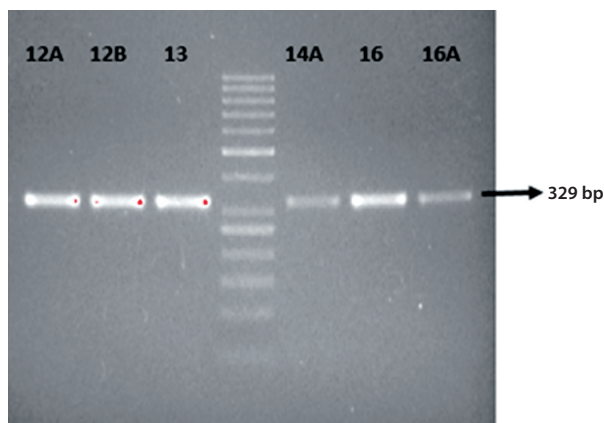


FIGURE 1. Examples of the visualized PCR products from sample numbers 12A, 12B, 13, 14A, 16, and 16A

ence of bacteria and bacterial biofilms is, however, not sufficient for the destruction of periodontal tissues, and the extent of bacterial accumulation is poorly correlated with the risk or severity of periodontitis [6]. The individual outcome will strongly depend on the host immune responses, which are related to factors, such as genetic predisposition [6-8]. One of the implicated genes in the pathogenesis of periodontitis is *MMP-8* (chromosomal location, 11q22.3), which encodes a proteolytic matrix metalloproteinase enzyme (MMP) that regulates the repair and breakdown of the connective tissue [9-11]. In inflammation, elevated levels of MMP-8 are found in the periodontal tissues, gingival crevicular fluid (GCF), and saliva resulting in the degradation of extracellular matrix, which contains type-1 collagen, a major component of periodontal tissues. MMP-8 is produced by infiltrating inflammatory cells, such as neutrophils, plasma cells, and macrophages. In addition, it is produced by resident cells, including gingival fibroblasts and epithelial cells [9-11].

The functional variation in the expression of regulatory genes is commonly due to single nucleotide polymorphisms (SNP), and polymorphisms of *MMP-8* (Figure 1) could be related to the risk of periodontitis by modification of the innate and adaptive immune responses [12, 13]. Significant associations between periodontitis and the -799 C/T (rs11225395) polymorphism of *MMP-8* have been reported [14-16]. Another known SNP occurs at +17 C/G (rs2155052) in the first exon [12]. There is limited information about the effect of this polymorphism on periodontal disease [14, 15], with none in Indonesia.

OBJECTIVES

This study aimed to address the impact of this polymorphism on the risk of periodontitis in the Indonesian population.

TABLE 1. Demographic and clinical data of the periodontitis and control groups

Parameter	Periodontitis	Control
Age range	25-65	19-61
Mean age \pm SD	44.7 \pm 8.0 y	28.8 \pm 9.8 y
Gender (M/F)	70/30	91/9
PD (mm, mean \pm SD)	4.8 \pm 1.3	< 3.0
CAL (mm, mean \pm SD)	5.7 \pm 1.6	< 3.0

PD – pocket depth, CAL – clinical attachment loss

MATERIAL AND METHODS

SAMPLES

The study was approved by the ethics committee of the Faculty of Dentistry, Universitas Indonesia, and conducted by using samples of DNA that were stored (at -20°C) at the Oral Biology Laboratory of the Faculty of Dentistry [17-19]. The DNA samples were originally extracted from peripheral blood of 100 patients with periodontitis and 100 healthy individuals without periodontitis. Patients were examined by a periodontics (B.S.) and diagnosed as periodontitis based on anamnesis and clinical examination (bleeding on probing [BOP] (+), periodontal pocket depth \geq 4 mm, and the presence of clinical attachment loss [CAL]) according to the latest classification of periodontal disease [20]. The background information on the subjects is shown in Table 1.

POLYMORPHISM GENOTYPING

Genetic analysis was conducted by PCR-RFLP, with PCR amplification of the *MMP-8* gene using the following primer sequences: forward 5'-CTG TTG AAG GCC TAG AGC TGC TGC TCC-3' and reverse 5'-CAT CTT CTC TTC AAA CTC TAC CC-3' from Holla (2012), resulting in a 329 bp PCR product. For these reactions, every PCR microtube had a total volume of 20 ml, containing 10 ml of KAPA TaqReadyMix PCR with dye (Roche-Kapa Biosystem, Indianapolis, USA; KAPA code, BK1004, Roche Cat. No 07958498001), 0.75 ml forward primer, 0.75 ml reverse primer, 7.75 ml ddH₂O, and 0.75 ml DNA template. The amplification process consisted of stages of initial denaturation at 95°C for 1 min, followed by denaturation at 95°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 30 s, which was repeated in 35 cycles; a final extension at 72°C was then performed for 5 min. The polymorphism was detected by digesting the PCR product with the DdeI restriction enzyme (Biolabs, Ipswich, England) for 16 hours or overnight at 37°C in a water bath. The RFLP product was then inactivated in a thermoblock at 65°C for 20 min. Both PCR and RFLP results (fragments)

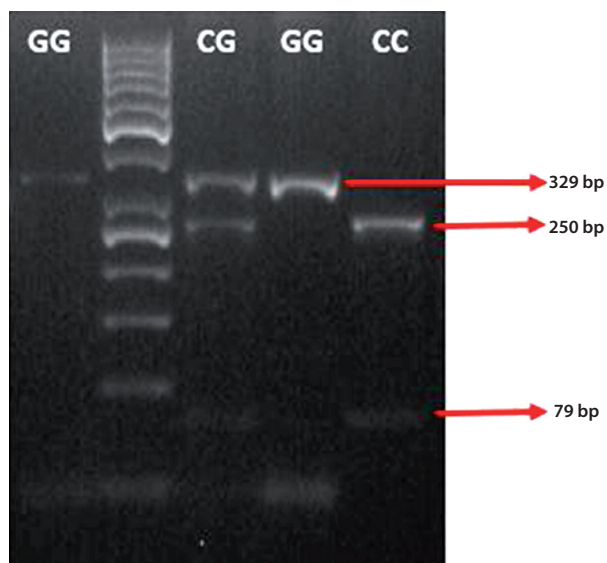


FIGURE 2. The visualization of RFLP products

were visualized using the GelDoc (Bio-Rad, Berkeley, California, USA) after electrophoresis in 3% agarose gel at 400 mA/80 V for 65 min. After RFLP, the GG genotype was shown as one 329 bp fragment, because the restriction enzyme DdeI did not recognize any restriction site. The CG genotype, with a restriction site recognized in one allele, appeared as three fragments that were 329 bp, 250 bp, and 79 bp in size. The CC genotype, with recognized restriction sites in both alleles, appeared as two fragments as 250 bp and 79 bp.

STATISTICAL ANALYSIS

Data analysis was conducted using the statistical package SPSS Ver22 (IBM, New York, USA) to analyze the distributions of the +17 C/G polymorphism of the MMP-8 gene in the periodontitis and control samples. The risk of periodontitis was evaluated using the χ^2 test and logistic regression (odds ratios).

RESULTS

Examples of the visualized 329 bp products after PCR amplification are shown in Figure 1, and examples

of the RFLP products are shown in Figure 2. The latter products appeared as a single 329 bp fragment in the case of the homozygote mutant GG genotype (lanes 1 and 4), as two 250 bp and 79 bp fragments in the homozygote CC genotype (wild type, lane 5), or as all three fragments representing the heterozygote genotype CG (lane 3).

Table 2 shows the genotype and allele distributions in the periodontitis and control groups. The wild type CC genotype appeared in 90% of the periodontitis group samples and in 95% of the control group samples, and the heterozygotic mutant genotype CG was the least common in both groups. The genotype distributions in both groups were inconsistent with the Hardy-Weinberg equilibrium, which states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences ($p < 0.0001$). The heterozygotic CG genotype appeared much less frequently than the GG genotype, but the number of samples with the CG genotype was too low for analysis. Table 2 demonstrates the corresponding allele distributions in the samples from the periodontitis and control groups.

The results (χ^2 test and odds ratios) showed no significant difference in the proportion of genotypes or alleles between the periodontitis and control samples. The raw odds ratios suggested that the periodontitis group was about a factor of two more likely to present with the polymorphic genotypes (and allele G), despite statistical significance.

DISCUSSION

The presence of MMP-8 is beneficial for normal tissues at low physiological concentrations. However, an increased expression of this gene leads to extracellular matrix degradation during periodontitis, particularly in periodontal tissues containing more than 60% of type 1 collagen [11, 13]. The MMP-8 polymorphism can, in principle, lead to a modified expression that could influence this process. The +17C/G polymorphism of MMP-8 in the exon 1 region results in a shorter form of the protein product, but the outcomes of the present study did not show a statistically significant impact on the risk of periodontitis. This may partly be attributed to the relatively low frequency of the mutant allele ob-

TABLE 2. Genotype and allele distributions in the +17 C/G polymorphism of MMP-8

Type	Periodontitis	Control	p-value	OR	CI (95%)
CC	90 (90%)	95 (95%)			
CG	2 (2%)	1 (1%)	0.283	2.111	0.695-6.416
GG	8 (8%)	4 (4%)			
C	182 (91%)	191 (95.5%)	0.111	2.099	0.919-4.792
G	18 (9%)	9 (4.5%)			

C – cytosine, G – guanine

served (Table 2; 9% in the periodontitis group and 4.5% in the control group). Therefore, an increase in the sample size might make a difference between the groups, appearing more significant (Table 2). However, the difference could be largely explained by the lower mean age of the subjects in the control group, and possibly by the fairly lower rate of smoking.

There are only a few comparable studies on +17 C/G polymorphism of MMP-8, but the results seem to be in general agreement with those in the present study, with no significant associations with chronic [14] or aggressive [15] periodontitis. The previous studies both from Czech and Turkish populations were conducted with 342 periodontitis subjects, 268 control subjects, and 100 periodontitis subjects, 167 control subjects, respectively [14, 15]. Moreover, none of those studies were conducted among Indonesian subjects. At first, we hypothesized that the origin of the population may influence the polymorphism of the +17 C/G polymorphism in MMP-8, but this was not the case here. In the present study, the diagnosis of periodontitis was made based on the latest consensus by the European Federation of Periodontology and the American Academy of Periodontology 2017, where there is no longer chronic or aggressive periodontitis, but rather a scale of staging and grading [20]. In addition, up till now, only few publications have implemented the latest consensus regarding the +17 C/G polymorphism in MMP-8.

The distribution of genotypes in the present study was not indicated as consistent with the Hardy-Weinberg equilibrium mainly because the number of samples with the GG genotype was larger than that with the CG genotype. However, the number of samples, particularly of the CG genotype, was insufficient for proper assessment in this respect; hence, the observation was considered as inconclusive.

Of the other known variants of the gene, the -799 C/T (rs11225395) polymorphism of MMP-8 has previously been shown to be associated with chronic and aggressive periodontitis [15, 16], whereas the -381 A/G (rs1320632) polymorphism of MMP-8 has not been shown to have a significant effect [14, 16]. The -799 C/T polymorphism apparently occurs about four times as often as the two other polymorphisms [12], including the one considered in the present study. This would decrease the sample size needed of the -799 C/T polymorphism to demonstrate significance between otherwise similar periodontitis and control groups. Some caution may be due because none of the MMP-8 polymorphisms has not been implicated in genome-wide association studies (GWAS) on periodontitis [21-25]. However, very few studies on the MMP-8 polymorphism related to periodontitis have been published in the literature; additional studies using larger samples from a wider range of the population are warranted.

Regardless of the significance of MMP-8 polymorphisms in periodontitis, the involvement of this gene

in periodontal tissue destruction can be used as an indicator of the disease in combination with other indicators [10, 11, 13]. A successful combination of the indicators can then provide the risk scores or models for predictive or status assessment of the onset and severity of periodontitis in an individual [26]. Apart from the required technical performance of such combined scores or models, there is also the issue of cost since dental services are generally the fourth most expensive health services in the developed world [2]. With decreasing costs of GWAS and other techniques used to quickly scan the multiple indicators, new cost-effective methods for clinical services may be expected in the future. This would support the development of a path toward more personalized provision of oral health services. The high costs of research and the limited facilities and infrastructure in the laboratory did influence this study. Nevertheless, we hope to conduct additional studies with the help of the government and the private sector in the future for the advancement of research in Indonesia.

CONCLUSIONS

The results showed no significant association between the +17 C/G polymorphism in MMP-8 and the risk of periodontitis among the Indonesian population. No change in the significance is expected even with a considerably larger sample.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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