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Research Article

POLYMORPHISM OF OSTEOPROTEGERIN GENE IN INDONESIAN MEN WITH PERIODONTITIS

AUERKARI EI[1]*, SAVITRI I.[1], MASULILI SLC[2], SUHARTONO A. W.[1]

[1]Department of Oral Biology, Faculty of Dentistry, University of Indonesia, Jakarta, 10430, Indonesia. [2]Department of Periodontics, Faculty of Dentistry, University of Indonesia, Jakarta, 10430, Indonesia. Email: eiauerkari@yahoo.com

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ABSTRACT

Objective: This work aimed to clarify the association of the severity of periodontitis and polymorphism in osteoprotegerin (OPG) (T950C) in Indonesian men.

Methods: For DNA extraction, blood serum samples were used from 100 consenting Indonesian males for whom also the status of periodontitis had been classified as mild, moderate, or severe. Polymerase chain reaction and restriction fragment length polymorphism techniques were applied to evaluate OPG (T950C) polymorphism, using Hind II restriction enzyme and electrophoresis on agarose gel to separate the indicative fragments.

Results: The genotype distribution of the OPG (T950C) polymorphism had an appearance of an increasing percentage of TT genotype (allele T) with increasing severity of periodontitis. The CC genotype was relatively rare (1%) in the tested Indonesian male population.

Conclusions: The results show no significant association between the severity of periodontitis and polymorphism of OPG (T950C) in Indonesian men.

Keywords: Periodontitis, Osteoprotegerin, Polymorphism.

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INTRODUCTION

Periodontitis is a complex multifactorial disease with high prevalence in Indonesia and in the world. According to the Indonesian national survey on domestic health (SKRT 2003), dental and oral diseases ranked first of the 10 groups of the most common diseases that people complained about, and of dental and oral diseases, periodontal disease was the most common complaint after caries. The survey of the following year (SKRT 2004) indicated a prevalence of 96.6% for periodontal disease [1]. In comparison, 50% of the population aged 16–80 years in Denmark had a healthy periodontium and 50% suffered from gingivitis and periodontitis in 1981. In America, 95% of the population aged 65 years or older is experiencing periodontal attachment loss in at least one side of the mouth. Among all age groups, men have higher levels of periodontal destruction than women [2]. In India, 76.7% of the population of outpatients aged 18–48 years showed periodontitis in 2008, and in Mexico, the prevalence of periodontitis was 62.7% in the male population in 2007 [3].

Periodontitis is an inflammatory disease in the supporting structures of the teeth caused by specific microorganisms and results in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both [4]. The interaction between host and bacteria of the oral cavity are very important to understand the pathogenesis of periodontitis. Initially, periodontopathic bacteria will attack the host, which then reacts with the immune response and can slowly damage the periodontium tissue through inflammatory processes that occur. The presence of subgingival bacteria alone is not sufficient to cause periodontal damage in most cases. Therefore, although the bacteria are initiating periodontitis, the amount of plaque and bacterial species are not always correlated with the severity of disease [5].

In addition to bacterial pathogens and various environmental factors (e.g., smoking and stress) influencing the pathogenesis of periodontitis, other such as genetic factors are also involved [6-8]. To fight bacteria, every person has an individual body response that will determine the susceptibility to periodontitis. Hence, the variants such as polymorphisms of the key genes controlling the host response in periodontitis may affect the susceptibility to the disease [9].

Pathophysiological periodontitis is characterized by alveolar bone resorption by osteoclasts. Formation of osteoclasts or osteoclastogenesis is controlled by three members of the tumor necrosis factors (TNF) and TNF receptor superfamily, namely receptor activator of nuclear factor-kappa-B (RANK), RANK-B Ligand (L), and osteoprotegerin (OPG). RANKL can activate the differentiation of precursor cells into osteoclasts, increase the activity of mature osteoclasts, and inhibit osteoclast apoptosis by binding to the functional receptor RANK. In contrast, OPG is a soluble receptor that can inhibit the interaction between RANK and RANKL. In patients with periodontitis, increased concentration of RANKL and decreased concentration of OPG have been typically detected in gingival crevicular fluid. OPG polymorphisms can be expected to affect the OPG structure and function, either directly through the functional efficiency of OPG or by changing the ratio of OPG/RANKL. However, the influence of variant alleles on the expression and biological activity of OPG in periodontitis are not well known [10-12].

This study aimed to clarify the relation of the OPG polymorphism (T950C) with respect to the severity of periodontitis in Indonesian men.

METHODS

Blood serum samples of 100 men with periodontitis were obtained from the archival material of the Laboratory of Biology, Faculty of Medicine, University of Indonesia [13-16]. These samples were stored in a freezer at −20°C and used for the extraction of DNA. The subjects had also been evaluated in terms of the severity of periodontitis and on this basis grouped into classes of mild (10 subjects), moderate (49 subjects), and severe (41 subjects) periodontitis. From the extracted DNA, a 147 bp fragment was amplified by polymerase chain reaction (PCR) using the following primer pair: Forward: 5'-GGG GGA TCC TTT CCG CCC A-3' and reverse: 5'-GTA TCG CCT GCC TTT GAT CAG T-3'. For this purpose, 1 µL of the genomic DNA was used for PCR amplification in a reaction mixture containing 10 µL KAPPA PCR mix and 2 µL of each primer. The reactions were performed in a Techne T-512 Thermal Cycler and

consisted of denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 30 s, annealing at 55.6°C for 30 s, and elongation at 72°C for 30 s, with a final extension at 72°C for 7 min. The amplification result is shown in Fig. 1. Restriction fragment length polymorphism (RFLP) technique was applied in a final reaction volume of 10 µL, using 0.1 µL of Hind II enzyme (5'.GTY/RAC. 3') and aliquot of PCR products, digested at 37°C for 4 h and 65°C for 20 min. The digested products were separated by 3% agarose gel electrophoresis mixed with 2 µL GelRed™ nucleic acid gel stain, ×10,000 in water. The OPG (T950C) genotypes were determined by comparing the RFLP band patterns with a 25 bp DNA ladder. The RFLP site is formed by a single base transition (T/C) of the OPG gene targeted as a Hind II restriction site. The PCR products of OPG (T950C) can appear in three possible combinations after cutting (Fig. 2). A double band of 110 bp and 37 bp suggests genotype CC, while the heterozygous genotype (TC) is indicated by bands of 147 bp, 110 bp, and 37 bp in size. A single band of 147 bp indicates the genotype TT (no cutting by Hind II).

RESULTS

Table 1 shows the distributions of genotypes and allotypes of OPG (T950C) polymorphism in all 100 male subjects. It is seen that the TT genotype (70%) dominates and CC is rare (1%), with corresponding dominance of allele T (84.5%). Table 2 presents the distribution of genotypes according to the severity of periodontitis. The TT genotype appears to become increasingly common from mild to severe periodontitis, and the TC genotype is correspondingly less frequent. Table 3 shows the distribution of alleles according to the severity of periodontitis. Allele T appears to become more frequent from mild to severe periodontitis, and allele C shows an opposite trend. However, the trends are not statistically significant.

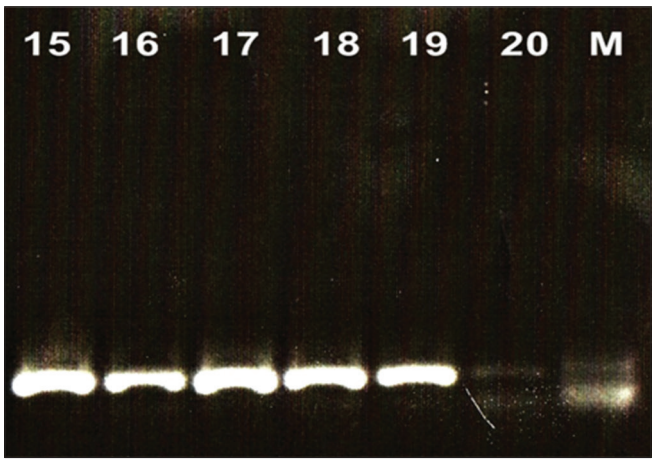

Fig. 1: Polymerase chain reaction fragment showing a single band of amplified osteoprotegerin (147 bp)

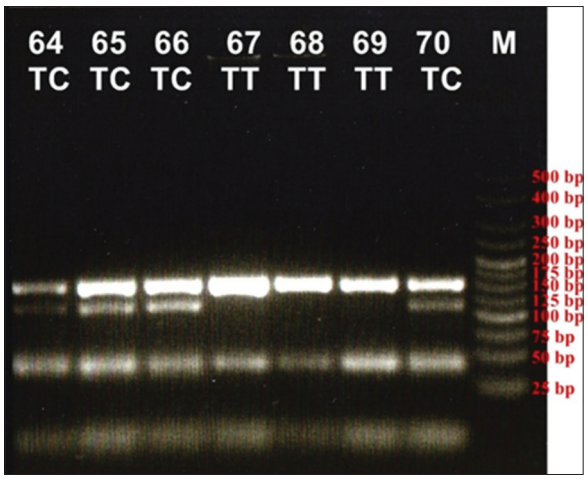

Fig. 2: Electrophoresis fragments from restriction fragment length polymorphism cutting, indicating TT genotype by a single band at 147 bp for samples 67, 68, and 69 and TC genotype by bands at 147 bp, 110 bp, and 37 bp for samples 64, 65, 66, and 70

DISCUSSION

The results suggest an increasing frequency of the TT genotype (T allele) of the OPG (T950C) polymorphism with increasing severity of periodontitis in Indonesian males. In case of mild periodontitis, one-half of the subjects were of the TT genotype but >80% in case of severe periodontitis. In principle, this could suggest some oral health benefits carried by the TC genotype, but it should also be noted that the CC genotype appears to be rare (1%) in the test population. This, in turn, may suggest a disadvantage carried by the CC genotype, unless the low frequency of CC is coincidental. For comparison, the distributions of OPG (T950C) genotypes are shown in Table 4, as determined for some other populations in the world. It is seen that the genotype distributions for some cases, notably Germany and perhaps Brazil, are similar or roughly similar to that observed in the present work. However, some others are clearly different, and it is not immediately clear why this should be the case. For example, the population of Germany is generally not expected to differ genetically much from that in Denmark, but by the reported distribution of OPG (T950C) polymorphism, there seems to be a remarkable difference.

Previously, Park *et al*. suggested that OPG polymorphism is significantly associated with chronic periodontitis [17]. However, several other studies have shown no association between chronic periodontitis and aggressive periodontitis with polymorphisms of OPG [18-22]. Considering the wide variation of the genotype distribution between populations, as indicated by Table 4, and the population-dependent prevalence of periodontal disease, it is likely that the strength of such an association, if present, varies between populations. In general, one would not expect that a single polymorphism of a single gene, although an important gene in the control of alveolar bone resorption, would alone show an overwhelming association to the pathogenesis of periodontitis that, after all, is well known to be a multifactorial disease. In the case of the present work on male Indonesian subjects, there was a clear trend of higher frequency of the genotype TT (allele T) occurring with increasing severity of periodontitis. Although not statistically significant, this trend may suggest a significant association for a larger sample population. Nevertheless, any association would be unlikely to be very strong for the tested polymorphism alone.

CONCLUSIONS

There was an increasing frequency of the TT genotype (T allele) of the OPG (T950C) polymorphism with increasing severity of periodontitis

Table 1: Genotype and allotype distribution of the subjects

Issue	Genotype				Allele		
	TT	TC	CC	Total	T	C	Total
Number	70	29	1	100	169	31	200
Frequency (%)	70	29	1	100	84.5	15.5	100

Table 2: Distribution of genotypes according to the severity of periodontitis

Genotype	Severity		
	Mild n (%)	Moderate n (%)	Severe n (%)
TT	5 (50.0)	31 (63.3)	34 (82.9)
TC	5 (50.0)	17 (34.7)	7 (17.1)
CC	0	1 (2.0)	0
Total	10 (100)	49 (100)	41 (100)

Table 3: Distribution of allotypes according to the severity of periodontitis

Allele	Severity		
	Mild n (%)	Moderate n (%)	Severe n (%)
T	15 (75.0)	79 (80.6)	75 (91.5)
C	5 (25.0)	19 (19.4)	7 (8.5)
Total	20 (100)	98 (100)	82 (100)

Table 4: Genotypes of OPG (T950C) in some populations

Population (n)	Genotype		
	TT (%)	TC (%)	CC (%)
Brazil (50)	82	12	6
Germany (90)	66	32	2
Slovenia (103)	25.2	53.4	21.4
Japan (361)	37.7	44.9	17.4
Denmark (266)	23.7	53.4	22.9
This work (100)	70	29	1

OPG: osteoprotegerin

in Indonesian males. However, the trend was not statistically significant for the tested sample size.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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