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Research Article

THE HUMAN BETA-DEFENSIN-1 LEVEL FROM SMOKERS WITH CHRONIC PERIODONTITIS

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ABSTRACT

Objective: The role of human beta-defensin-1 (HBD-1) as an antimicrobial peptide in a smoker's periodontitis is still unclear, this study aimed to analyze the association of the levels of HBD-1 in smokers and non-smokers with chronic periodontitis.

Methods: In total, 104 male subjects, 33-78 years old, were diagnosed to have chronic periodontitis in the Department of Periodontology, Oral Disease Special Clinic of the University of Indonesia. This cross-sectional study included clinical and laboratory examination. The data collected included those from anamnesis, clinical examination (oral hygiene index Siller, pocket depth, clinical attachment level), and smoking status. The samples were stored at −20°C until testing for HBD-1 level by enzyme-linked immunosorbent assay.

Results: The median (min-max) HBD-1 level in the group of mild-moderate chronic periodontitis was 57.61 (0.87-343.58) pg/ml and in the group of severe periodontitis 15.27 (0.94-198.03) pg/ml (p=0.087). The median (min-max) HBD-1 level in non-smokers with chronic periodontitis was 27.82 (0.92-200.58) pg/ml, and in smokers 25.04 (0.87-343.58) pg/ml (p=0.457).

Conclusion: There were no significant differences in the HBD-1 levels in subjects with chronic periodontitis regardless of the smoking status or severity of the disease.

Keywords: Periodontitis, β-defensin, Smokers.

INTRODUCTION

Based on the National Health Survey in Indonesia 2010, periodontal disease took the second place of the dental health problems, with 42.8% of the population in Indonesia [1]. Chronic periodontitis occurs as a result of the host response to the aggregation of bacteria on the tooth surface. The disease is characterized with absolute periodontal pockets and alveolar bone damage [2]. Any involvement of systemic factors such as diabetes, smoking, and stress will accelerate the progress of periodontal disease because of changes in the host response to the accumulation of plaque [2].

Smoking is a known risk factor of periodontal disease [3]. Smoking periodontal patients show a more extensive alveolar bone and tooth loss compared with non-smoking patients [4]. The severity of the disease depends on the intensity and duration of smoking [4]. Cigarette smoke will modulate the immune response of the host to the bacterial plaque, indicated by aggressive periodontal tissue destruction [4].

In response to the invasion of bacteria, the body will synthesize pro-inflammatory cytokines such as interleukin (IL)-1, IL-8, tumor necrosis factor-α, and also antimicrobial peptides such as human beta-defensin-1 (HBD-1) [4]. HBD-1 is an antimicrobial molecule that acts in the innate immune system and provides signaling for the adaptive immune system [5,6]. The role of HBD-1 is to pull the immature dendritic cells, T cells (memory) CD4/CD45RO, and CD8 T cells by binding to chemokine receptors, namely, CCR6 that will facilitate the destruction of Antigen (Ag) by making a "defensin-Ag" complex [7]. The expression of HBD-1 has been seen in the gingival epithelium, buccal mucosal epithelium, pulp and also in glandular salivary tissue [8]. Many studies suggest that HBD-1 is keeping the homeostasis of microbial pathogens by preventing bacterial colonization and viral infection [8]. HBD-1 is expressed in infected epithelial tissue, but also plays a role in wound healing and tumor suppression [9]. HBD-1 is expected to be a potential tool for therapeutic approaches and to stimulate wound healing [9]. In particular, HBD-1 has a large effect on the proliferation and differentiation of osteoblasts. HBD-1 has also been suggested as a vaccine against HIV-1 as well as a diagnostic biomarker for cancer [9]. There is a controversy in the expression of HBD-1 in inflamed gingival tissue and healthy tissue [6]. Immunohistochemical testing shows the presence of HBD-1 on inflamed marginal and epithelial sulcus exposed to dental microbial plaque [6]. There was an improvement regulation of HBD-1 in inflamed epithelium [10].

The present research aimed to analyze the association of the levels of HBD-1 in smokers and non-smokers with chronic periodontitis. Subjects with chronic periodontitis and smoking will show symptoms of inflammation in periodontal tissues, as well as increased levels of HBD-1 in the oral epithelial lining of the periodontal tissues. The selection of sampling to detect HBD-1 from the gingival epithelium was done from periodontal lesions on mucosal areas exposed to tobacco smoke. As the treatment of periodontal disease in smokers can be challenging, it is worthwhile to explore the potential benefits of HBD-1 as inflammation biomarker and antimicrobial agent.

METHODS

Subjects

In total, 104 male subjects, 33-78 years old, were diagnosed with chronic periodontitis in the Department of Periodontology, Oral and Dental Special Clinic of the University of Indonesia. The subjects had signed the written declaration of informed consent and had not received any periodontal treatment before. An anamnesis, clinical examination (oral hygiene index Siller, pocket depth, clinical attachment level (CAL)), and survey of smoking status were conducted on each subject. Tissue sampling is taken from the gingival epithelium. It was following the procedures with ethical approval from the Ethics Committee of the Faculty of Dentistry, University of Indonesia. The sampling was conducted before the subjects received any kind of periodontal therapy, from the pockets of chronic periodontitis with pocket depth <5 mm

for the mild-moderate periodontitis group, and pocket depth ≥5 mm for the severe periodontitis group. Then, the samples were stored into Eppendorf tubes containing sterile phosphor buffered saline. The samples were stored at −20°C until enzyme-linked immunosorbent assay (ELISA) testing.

ELISA protocol

About 76 samples were selected by consecutive sampling. HBD-1 ELISA kit, pink-ONE, KOMA BIOTECH INC (Korea) was used in this study. For the ELISA procedure, the samples were thawed at room temperature. Each 100 mL of samples and standards were inserted into the well plate and incubated at room temperature for 2 hrs, and then aspirated well to dispense fluid and to wash the well. A 100 mL batch of diluted detection antibody (0.5 mg/ml) was added per well and incubated at room temperature for 2 hrs, aspirated back and rinsed. Add 100 mL diluted color development enzyme (1:20 dilution) per well, and incubate for 30 minutes at room temperature (37°C for 30 minutes). Aspirate back and the rinse. For the last, added 100 mL pink-ONE TMB color development reagent to each well, incubated for 1-8 minutes to form a color changing, add 100 mL stop solution to stop the color changing happens. Then, well plate is read by the plate reader at a wavelength of 450 nm. Once the well plate is inserted into the plate reader (Accu Reader) and it will show the optical density and the concentration of HBD-1 from each well.

Statistical analysis

Data were analyzed with SPSS version 20. The Kolmogorov-Smirnov was used to test normality. For normally distributed data, the independent *t*-test can be applied; otherwise, the Mann-Whitney test is selected.

RESULTS

All research subjects were diagnosed to have chronic periodontitis. The age is ranging from 33 until 78 years old, with a mean of 51.17 years and standard deviation of 9.8 years.

Table 1 shows a balanced number of subjects between mild-moderate and severe levels of periodontitis. Considering the smoking status, the number of smokers is slightly higher than non-smokers. The distribution of mean CAL on clinical examination was 5.54±2.32 mm with a minimum of two and a maximum of 12 mm.

Table 2 shows the results of normality testing. The distribution of HBD-1 in the mild-moderate group appears normal (p=0.09), but the levels of HBD-1 in severe periodontitis or the levels of HBD-1 in smokers or non-smokers are not normally distributed. Nonparametric Mann-Whitney test was applied to determine the association of the levels of HBD-1, severity of periodontitis, and smoking status (Tables 3 and 4).

The mean levels of HBD-1 in mild-moderate periodontitis group were higher (69.97±71.76 pg/ml) than the severe periodontitis group (40.50±53.37 pg/ml), although the difference was not indicated as statistically significant.

The mean and median levels being fairly similar, it appears that there is no significant difference in the levels of HBD-1 between smokers and non-smokers (p=0.457).

DISCUSSION

In this study, no significant difference was found in the levels of HBD-1 between mild-moderate periodontitis and severe periodontitis groups (p=0.087). This is consistent with the findings of Bissell *et al*. (2004) suggesting that the differences in the levels of HBD-1 between healthy gingival and pathologic conditions were not significant [11]. A possible reason is that HBD-1 can activate the expression of pro-inflammatory mediators in infected areas, whereas in healthy areas there is also a pro-inflammatory mediator expressed as a growth factor. Another reason for the HBD-1 levels appearing not significantly different could be the additional genetic variation of the HBD-1 levels. Yet another possibility for the pathological condition is that HBD-1 is degraded by cathepsin cysteine proteases to become inactivated. When an infection occurs, then an increase of cathepsin will cause the degradation of HBD-1 directly, leading to bacterial colonization and infection. Bissell *et al*. (2004) also noted that there is no association of the expression of HBD-1 with age and sex [11]. On the other hand, the results of Lu *et al*. (2004) showed that increased HBD-1 expression in the walls of the pocket compared to healthy tissue (p<0.05) [6]. Inflammatory mediators would affect the expression of HBD-1. Increased IL-4 and IL-13 levels will inhibit HBD-2, whereas IL-1 will stimulate both HBD-1 and HBD-2 [6]. Sengul *et al*. (2007) found low regulation of HBD-1 in gingivitis and high regulation of HBD-1 in chronic periodontitis [12]. In this study, the level of HBD-1 was positively observed in all samples, but not comparing the condition of gingivitis to periodontitis.

The potential of HBD-1 as a biomarker of periodontal inflammation is still not fully supported by the evidence. Winter *et al*. (2012) argue that the HBD-1 will increase the proliferation and migration of keratinocyte cells and has a positive effect on wound closure [9]. Studies by Bissell *et al*. (2004) found elevated levels of HBD-1 in healthy tissue (p>0.50) [11], while Sengul *et al*. (2007) found no increase of

Table 1: Subject distribution based on severity and smoking status

Subject	n (76)	Percentage (100)
Periodontitis		
Mild-moderate	38	50
Severe	38	50
Smoking status		
Smokers	39	51.3
Nonsmokers	37	48.7

Table 2: Normality test on the level of human beta-defensin-1, severity of periodontitis, smoking status

Variable	p
Human beta-defensin-1 level	
Mild-moderate	0.09*
Severe	0.00
Human beta-defensin-1 level	
Smokers	0.00
Nonsmokers	0.00

Kolmogorov-Smirnov test; *p>0.05: Normal data distribution

Table 3: Median, mean, and standard deviation of the level of human beta-defensin-1 with the severity of periodontitis

Human beta defensin-1	n	Median (pg/ml) (minimum-maximum)	Mean±SD (pg/ml)	p
Chronic periodontitis				
Mild-moderate	38	57.61 (0.87-343.58)	69.97±71.76	0.087
Severe	38	15.27 (0.94-198.03)	40.50±53.37	

Mann-Whitney U-test; p<0.05 significant difference, SD: Standard deviation

Table 4: Median, mean and standard deviation of the level of human beta-defensin-1 with smoking status

Human beta-defensin-1	n	Median (pg/ml) (minimum-maximum)	Mean±SD (pg/ml)	p
Chronic periodontitis				
Smokers	39	25.04 (0.87-343.58)	52.12±69.30	0.457
Nonsmokers	37	27.82 (0.92-200.58)	58.51±59.90	

Mann-Whitney U-test; p<0.05 significant difference, SD: Standard deviation

HBD-1 in chronic periodontitis group compared with control group ($p<0.001$) [12]. Lu *et al.* (2004) argue that HBD-1 is increased in the epithelium pocket compared with healthy tissue [6]. The differences in outcome are likely to arise from the differences of the severity of periodontitis, research methods and possible genetic variations related to the expression of HBD-1. This study could not confirm the HBD-1 as a biomarker of inflammation because this study did not compare the pathological condition with a healthy one. The study is limited only to analyze the association of HBD-1 with the severity of periodontitis and smoking status.

In this study, there was no significant difference in the levels of HBD-1 in smokers and non-smokers ($p=0.457$). This is not in accordance with the results of *in-situ* studies by Mahanonda *et al.* (2009) who found that cigarette extracts can reduce the expression of HBD [4]. Cigarette smoke is known contain carbon monoxide which will directly reduce the oxygen levels. The body will compensate the oxygen drop by vasoconstriction of blood vessels, which in turn will reduce the host response to inflammation. Smokers have been shown to have a risk of alveolar bone loss more severe than non-smokers [13]. Thaper et al. (2016) in his discussion also support that smoking could increase the risk of periodontitis about 10 times greater in systemic disease. [14]

CONCLUSION

In this study, no significant association was found between the levels of HBD-1 and the severity of chronic periodontitis or the smoking status. The author found a slightly higher level of HBD-1 in non-smokers than in smokers, but it was not statistically significant. The HBD-1 levels in mild-moderate periodontitis group were also higher than the severe periodontitis group, but this result was again not statistically significant.

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