Dental Journal
Majalah Kedokteran Gigi
Vol. 42. No. 4 October–December 2009

Research Report

Detection of aggressive periodontitis by calprotectin expression

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ABSTRACT

Background: *Calprotectin is a calcium-binding protein expressed by neutrophil, monocytes, gingival keratinocytes, and oral epithelial cells. The concentrations of calprotectin increase in plasma, urine and synovial fluid of patients with inflammatory diseases. This protein is known as a marker for periodontal diseases and is detected in gingival crevicular fluids.* ***Purpose:*** *This study was aimed to investigate the detection of inflammation on the aggressive periodontitis by calprotectin expression.* ***Method:*** *The gingival crevicular fluids were taken from five aggressive periodontitis patients and five healthy subjects by using sterile paper points. Calprotectin expression was analyzed by ELISA technique.* ***Result:*** *The results showed the significant difference in calprotectin expression between subject with aggressive periodontitis and healthy subjects $p = 0.002$ ($p < 0.05$).* ***Conclusion:*** *It was concluded that the calprotectin expression on the aggressive periodontitis patients may be useful for evaluation the progression of inflammation in periodontitis.*

Key words: *Calprotectin, aggressive periodontitis, gingival crevicular fluids*

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INTRODUCTION

Aggressive periodontitis is a type of periodontitis attacking adult people in the age of 20 to 35 years old. The character of aggressive periodontitis is the fast and severe damage of periodontal tissue with gingival inflammation, bleeding, and exudation followed by the lost of alveolar bone in months, but it does not correspond with the amount of plague and calculus.[1,2]

Aggressive periodontitis can occur locally or generally. The special mark of local aggressive periodontitis is started from the puberty period with the damage of the first insisive and molar alveolar bone. The damage of alveolar bone in general type occurs in almost all teeth. During the active phase, the development of aggressive periodontitis is marked by the inflammation of development gingival tissue with the proliferation in margin, and then is followed with the inactive phase, or even stops with or without gingivitis history.[3]

Aggressive periodontitis is actually caused by the growth of negative periodontal pathogen in its gingival cycle followed by the inflammatory respond of immunity in the vulnerable host. Thus, the patient of aggressive periodontitis has sensitive aspect in responding immune such as abnormality of neutrophile cells in the terms of chemotaxis, phagocytosis, adherence, and bactericide activities.[1]

Neutrophile is very important during inflammation, especially in the mechanism of immunity towards the infection of periodontophatogenic bacteria. The function of neutrophile is to protect the integrity of periodontal tissue. However, in the severe periodontal disease the capability of neutrophile seems to decrease in controlling pathogenic bacteria.[4] Cytoplasm of neutrophile actually contains many anti-microbes inside its granules, myeloperoxides, defensin, elastase, proteinase, cathepsin G, azurosidin, laktoferin, lisosom, and calprotectin. The concentration of calprotectin in the cytoplasm of neutrophile is about 40–60%.[5]

Calprotectin is protein binding with calcium contained in neutrophile, monocyte, keratinocyte cells, and epithelial cells with the molecular mass about 36.5 kDa. Calprotectin actually is also known as macrophage migration inhibitory factor-related protein 8 and 14 (MRP8 and MRP14), cystic

fibrosis antigen, calgranulin A and B, and S1000A8 and S1000A9. In healthy gingival epithelia, calprotectin is located in stratum spinosum cells, and during inflammation calprotectin can also be detected in stratum spinosum and granulosum cells.[6,7]

Calprotectin is produced by neutrophile, monocyte, macrophag, keratinocyte cells and epithelial cells. The concentration of calprotectin increases in patients with infection, tumor, and allergic reaction. The concentration of calprotectin located in plasma, urine, and synovial fluids of patients with inflammatory diseases, such as pneumonia, meningitis, urine duct infection, rheumatoid arthritis, and cystic fibrosis. The concentration also increases compared with healthy patients.[8]

In addition, the in vitro study shows that calprotectin has anti-microbe function, bacteriastatics and fungistatics with Minimal Inhibitory Concentration (MIC) that is similar with some antibiotics. The mechanism of this action is actually caused by the increasing of bacteria binding zinc needed for proliferation.[9] Calprotectin, therefore, is not only found in calculus, but also in gingival cervicular fluid in which its concentration in the periodontal disease patients is higher than that in healthy patients.[10]

The fast damage occurred in patients with aggressive periodontitis is actually related more with the chemotaxis dysfunction of neutrophile and monocyte. Since the normal role of neutrophile and monocyte in producing possibly has been changed, the chemotaxis dysfunction of neutrophile can get problem causing the susceptibility of immune system towards periodontophatic bacteria.[7]

Calprotectin is secreted by neutrophile during the process of inflammation, and it also has an important role in the mechanism of body immune towards periodontal inflammation. The reason is because the concentration of calprotectin that detected in the gingival crevicular fluids can be potentially used as the maker of the clinical inflammation of periodontal disease. The concentration of calprotectin in the gingival crevicular fluids can also reflect the severity of the inflammation in patients with periodontal disease.[7,8] Thus, in order to analyze the mechanism or pathogenesis of this disease, the concentration of calprotectin showing the degree of the inflammation in those patients with aggressive periodontitis must be examined.

The aim of the study was to detect the inflammation on patients with aggressive periodontitis by calprotectin expression in the gingival crevicular fluids.

MATERIAL AND METHOD

The type of the study conducted was observational with laboratory approach. Before the study was conducted, ethical clearance must be set up and approved by Ethic Commetee of Health Institusional. Five patients with aggressive periodontitis and five healthy patients had also approved informed consent. The criteria of sample, moreover, were that they must be in the age of 20–35 years old; without any systemic abnormality; have no smoking habit; using no mouthwash or antibiotics minimally for about the last six months; not under periodontal treatment minimally for about the last six months; and that they must not have pregnancy or periods.

In addition, the dental element of aggressive periodontitis patients suffering inflammation (diseased site) had pocket with ≥ 6 mm depth, while the healthy dental element (healthy site) of the healthy patients had light pocket (≤ 3 mm). Firstly, teeth were cleaned by sterile cotton rolls in order to clean supragingival plague. Then, gingival crevicular fluids was taken. After that, the sterile paper point was put into the pocket and abandoned for about 30 seconds. The paper point then was put into 0.5 ml eppendorf tube, covered and sealed with paraffin tape, put into ice box, and stored in deep freezer –30^{o} C in order to examine the concentration of calprotectin.

Afterwards, calprotectin in gingival crevicular fluids was examined with Enzyme Linked Immunosorbent Assay (ELISA) method. The sample then was dissolved by using extraction solution and had got centrifugation with 2500 rpm for 15 minutes in the temperature of 4 degrees Celsius. The sample then was diluted about 50–500 times. For optimization, it can also be done by using diluent solution, and by having centrifugation with 2500 rpm for 15 minutes in the temperature of 4 degrees Celsius. The sample then was applied in 96 wells of microtiter plate coated by specific rabbit antibody for calprotectin. The application of the sample into each plate was about 50 µl. Those plates then were covered by aluminium foil paper and incubated for 45 minutes in the room temperature.

After that, the fluid was discharged by suction using washing buffer fluids. Then, they were added by conjugated antibody enzyme, incubated again for 45 minutes at temperature of a dark room, and washed about 5 times. Substract solution enzyme was added into each of those wells for about 100 μl in the room without light for about 20 minutes. The result of the average calprotectin concentration then was measured with optical densities of ELISA reader 405 nm (BioRad Bench Mark). One hundred μl stop solution (NaOH) was also added into each of those wells.

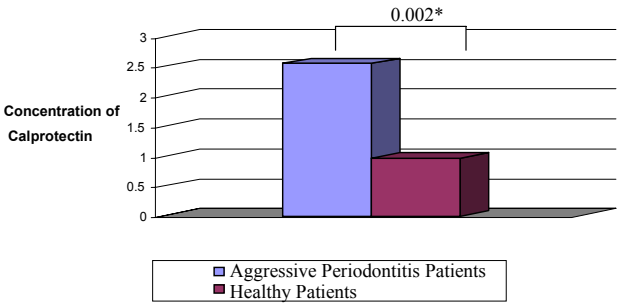
The data then was analyzed with t-test in order to find the difference of the calprotectin concentration between the patients with aggressive periodontitis and the healthy patients with the reliability degree 95%.

RESULT

The respondents of the study were 10 people (21–35 years old) divided into 2 groups, with the average age of the subjects 29.3 years. The result of the study showed that the average and standard deviation of calprotectin concentration in the aggressive periodontitis patients was about 2.55 ± 0.44, meanwhile in the healthy patients was about 0.96 ± 0.6 (Figure 1), so that concentration of

calprotectin in the aggressive periodontitis patients was higher than that in the healthy patients.

The result of t-test analysis showed F = 0.002 and sig = 0.965 (> 0.05). It indicated that both groups have the same variant. The result of the analysis also showed that there was a significant difference of calprotectin expression in the aggressive periodontitis patients and the healthy patients with t = 4.73 and sig (2-tailed) = 0.002 (p < 0.05).



Note* Significance p < 0.05

Figure 1. The average of calprotectin concentration based on the group.

DISCUSSION

The main cause of aggressive periodontitis was the infection of periodontophatogen bacteria in subgingival area. The species of bacteria colonizing in periodontal pocket area must adhere to the surface in order to prevent fluids from gingival crevicular fluids. The bacteria then might infuse into the main tissue through lesion of the gingival crevicular epithel or pocket, and infuse into the gingival tissue. Another route of the bacteria in invading the tissue was by direct penetration into the main epithel or bound tissue. *P. gingvalis*, *P. intermedia*, *A. actinomycetemcomitan*, *F. nucleatum*, and *T. denticola bacteria*, could invade directly to the cells of the main tissue.[11]

The previous study actually has successfully identified eight bacteria in subgingival plague, *A. actinomycetemcomitan*, *T. denticola*, *F. nucleatum*, *P. intermedia*, *P. gingivalis*, *E. corrodens*, *T. forsythia*, and *C. rectus* in the bleeding area, and only three of them are very patogen in aggressive periodontitis patients, which are *A. actinomycetemcomitan*, *P. gingivalis*, and *P. intermedia*.[12]

P. gingivalis and *P. intermedia* bacteria dominate periodontal pocket area of aggressive periodontitis patients, i.e. about almost 85%, compared with that of chronic aggressive periodontitis patients, about 65%. Those anaerob negative gram bacteria are able to invade mucosa barrier and infuse into epithelial cells causing destruction in periodontal tissue because of the product of bacteria, lipopolysaccaride.[13]

The previous study conducted by Kido *et al.*,[14] also showed that lipopolysaccaride of periodontopathic bacteria can cause the in vitro discharging of calprotectin from neutrophile. However, the stimulation of lipopolysaccaride from *P. gingivalis* can also cause the discharging of calprotectin increasing about 15 times compared with the control one. The discharging of calprotectin is actually caused by LPS and inflammatory cytokine, such as TNF-α, IL-1β, and PGE_2. TNF-α and IL-1β cause the significant production of calprotectin after 24 to 72 hours in monocyte. The concentration of calprotectin in gingival crevicular fluids relates with the clinical indicator, especially the depth of pocket marker biochemical marker, TNF-α, IL-1β, and PGE_2 in the periodontal disease.

Calprotectin has chemotaxis, adhesion, regulation, and migration activities of neutrophile and monocyte, and also has important role in body immunity as natural immune system for periodontal disease.[15] The concentration of calprotectin increases 3–4 times in periodontitis patients compared with that in the healthy patients. The concentration of calprotectin in the pocket with more than of 7 mm depth becomes 2400 ng/μl. It showed that there was significantly positive correlation between the concentration of calprotectin and the depth of the pocket.[10]

The height of the calprotectin concentration in gingival crevicular fluids of periodontitis aggressive patients is caused by the increasing number of periodontopathic bacteria in the periodontal pocket, so lipopolysaccaride of periodontopathic bacteria can stimulate the secretion of calprotectin from neutrophile cells.[16]

When calprotectin is discharged from neutrophile through the signal line of lipopolysaccaride, lipopolysaccaride interacts with some receptors, such as: CD14 and Toll-like receptor (TLR) that activate the function of nuclear factor-κB (NF-κB), so it can cause the process of transcription in the nucleus and the process of translation in the ribosom, which then it is synthesized into calprotectin.[8,17]

The concentration of calprotectin in gingival crevicular fluids increases about 12 times compared with that in chronic periodontitis patients and in the healthy patients in the longitudinal study of aggressive periodontitis patients.[16] Calprotectin is discharged through physiologic mechanism during the lifespan of neutrophile cells, in which there are more than 93.2% of cell viability after it is incubated with LPS P. gingivalis.[17]

Calprotectin can also be secreted by neutrophile in extracellular way as the result of the cell damage or death. The increasing concentration of calprotectin in extracellular fluids is considered as the result of the active or passive secretion when neutrophile is accumulated in local inflammation.[18] Calprotectin is actually considered as chemotaxis factor that has a role in early immune of an inflammation. With the stimulation of LPS calprotectin will be discharged and then will cause migration from neutrophile to inflammatory area.[19]

The susceptibility of the immune system of periodontal tissue towards periodontophatic bacteria in the aggressive periodontitis patients is caused by the functional alteration of chemotaxis and phagocytosis from neutrophile. The functional damage of phagocytosis occurs at the phase in

which bacteria adhere on the surface of neutrophile and then are internalized.[1] The concentration of calprotectin in gingival crevicular fluids also relates with clinical indicator, the depth of probing and Bleeding On Probing (BOP) in which the concentration of calprotectin is higher in the positive BOP than that in the negative one.[14]

Based on the result of this study, it can be concluded that calprotectin expression in the aggressive periodontitis patients was higher than that the healthy patients. The infection of periodontophatic bacteria through lipopolysaccaride could also cause the producing of calprotectin as the factor of chemotaxis which has a role in margination and initiation of neutrophile cells into the infection area. The high calprotectin expression in gingival crevicular fluids then reflected the degree of gingival inflammation in the aggressive periodontitis patients. Therefore, the concentration of calprotectin in gingival crevicular fluids could be used to detect the early symptom of an inflammation in periodontal disease.

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