THE RELATIONSHIP BETWEEN NEUTROPHIL ELASTASE LEVEL AND PERIODONTAL TISSUE DAMAGES AT GINGIVITIS AND PERIODONTITIS

(HUBUNGAN KADAR NEUTROFIL ELASTASE DENGAN KERUSAKAN JARINGAN PERIODONTAL PADA GINGIVITIS DAN PERIODONTITIS)

Nila Kasuma

Oral Biology Department
Faculty of Dentistry, Universitas Andalas
Jl. Perintis Kemerdekaan No.77, Padang-West Sumatra 25121
Email: nilakasuma@dent.unand.ac.id

Abstract

Periodontal disease consists of gingivitis and periodontitis. They occur due to the interaction between bacterial antigens and the host tissue hence neutrophil is activated, antibody is produced, and bone resorption is occurred. Enzymatic changes to diagnose periodontal disease index (PDI) based on biomarker measurement scale is useful for an accurate diagnosis and a successful measurement of periodontal disease therapy. Gingival crevicular fluid (GCF) was selected as the biological medium. This research aims to analyze the relationship between neutrophil elastase level and periodontal tissue damages at gingivitis and periodontitis. The study involved 66 people as samples with 22 healthy samples, 22 mild gingivitis samples and 22 early periodontitis samples. Enzyme level studied was tested using ELISA technique. In this cross-sectional study, neutrophil elastase enzyme level was compared between healthy, mild gingivitis and early periodontitis sample groups. Data analysis was performed using ANOVA test. The average NE level based on PDI group is $\bar{x}$± SD 2.65 ng/dl ± 1.28 in healthy sample group. There is an increase by 2.3 times in mild gingivitis sample group where NE level is$\bar{x}$± SD 6.27 ng/dl ± 1.13.While, the light periodontitis group has NE level of $\bar{x}$± SD 9.38 ng/dl ± 1.06 which equals to 3.5 times increase from the healthy sample group. This research concludes that the rise of neutrophil elastase level in gingival crevicular fluid is related to the severity of gingivitis and periodontitis diseases.

Keyword: Gingivitis, Periodontitis, Neutrophil Elastase

Abstrak

Penyakit periodontal terdiri dari gingivitis dan periodontitis, yang terjadi karena interaksi antigen bakteri dengan host-jaringan sehingga mengaktivasi neutrofil, produksi antibodi, dan resorpsi tulang. Perubahan enzimatik Periodontal yang memiliki skala ukur yang diharapkan dapat menjadi penanda pada penyakit Periodontal. Media biologis yang dipilih yaitu cairan sulkus gingiva (GCF). Pada penelitian ini melibatkan 66 subyek penelitian yaitu dengan 22 orang sehat, 22 orang gingivitis ringan, 22 orang periodontitis awal. Kadar enzim yang diteliti diuji dengan menggunakan teknik ELISA. Pada penelitian cross sectional membandingkan kadar enzim netrofil elastase pada sampel sehat, gingivitis ringan dan periodontitis awal pada setiap kelompok. Analisis data dilakukan dengan cara univariat untuk mendeskripsikan masing-masing variable, untuk melihat distribusi normal ($p > 0.05$) dilakukan Kolmogorov Smirnof Test. Apabila terdistribusi normal dilakukan uji ANOVA. Rata-rata kadar NE berdasarkan kelompok PDI pada kelompok sehat dengan $\bar{x}$± SD 2,65 ng/dl ± 1,28 dan pada gingivitis ringan naik 2,5 kali dibanding kondisi sehat dengan $\bar{x}$± SD 6,27 ng/dl ± 1,13, sedangkan pada peridontitis ringan naik 3,7 kali $\bar{x}$± SD 9,42 ng/dl ± 1,06. Penelitian ini menyimpulkan bahwa peningkatan kadar neutrofil elastase dalam gingival crevicular fluid berhubungan dengan tingkat keparahan penyakit gingivitis dan periodontitis.

Kata kunci: Gingivitis, Periodontitis, Neutrofil Elastase

INTRODUCTION

Periodontal diseases are defined as diseases in tissues that support the teeth (periodontal) which are initiated by bacteria. It is marked with inflammation and bone loss. Bacterial infection is a primary etiology, but it is not enough to initiate the growth of disease. Bacterial factors can stimulate local inflammation and activate immune system[1].

Interaction between host tissue and bacteria causes periodontal disease[2]. The responses from host either systemic or local are caused by the interaction between bacteria antigen in sulcus region and gingiva tissues which activates neutrophil, antibody and the loss of alveolar bones[3]. Neutrophil acts as host defence to pathogenic bacteria invasion. It is produced in the bone marrow and matured when released to the circulation which functions as cellular defence in the first line[4].

Periodontal enzymatic change with a scale of measure is expected to be a marker for periodontal diseases. This condition can be analyzed to have diagnosis based on the severity level of disease. It shows whether there are periodontal pathogens, gingiva and periodontal inflammations, host immune inflammation response to pathogen species and periodontal tissue damages. Gingival crevicular fluid (GCF) was selected as the biological medium. It is found in the V-shape gingival sulcus in the human gums which clinically stated as healthy. GCF is inflammatory exudates in microcirculation gingiva which crosses inflamed periodontal tissues as a local inflammation reaction[5]. The fluid acts as a defence mechanism that involves washing, protective cells and enzyme production. Cytokines pro inflammation and anti inflammation is identified in GCF. Its flow and the total transmigration of leukocyte will reach the maximum from 6 until 12 days after gingivitis initial lesion[6,7].

Gingival crevicular fluid (GCF) plays a role as a defence mechanism which reflects host responses to periodontal pathogen antigen bacterial and the growth of disease depends on host responses. Enzyme level measurement in GCF is a good method to diagnose patients with periodontal disease.

Periodontal disease involves a few enzymes that may destroy soft tissues which causes gingival inflammation to bone damages. One of them is Neutrophil Elastase (NE). Neutrophil is the main leu kocyte in gingiva sulcus due to the response towards bacterial biofilm. The decrease and rise of neu trophil affect the condition of periodontal tissue. Neutrophil will form NET (Neutrophil Extracellular Traps) which is useful to catch pathogen and release NE to inflamed region. However, when the equilibrium is disturbed, neutrophil will undergo repetitive cycle in which IL-17 is extracted in chronic inflammation areas to produce Th17 lymphocyte cells. This will result in the cumulation of neutrophil in that region and cause a prolonged inflammation[27]. Foreign materials phagocytosis and degradation processes are done by NE. This is aligning with NE functions as hydrolytic enzymes. ELA2 is the other name for neutrophil elastase. During inflammation process, neutrophil elastase is excreted by neutrophil and macrophage[4].

Neutrophil elastase is also one of antimicrobial mechanisms of neutrophil to destroy periodontal pathogen bacteria, Neutrophil elastase is excreted in neutrophil into phagolysosome in around microorganism therefore the pathogenic bacteria are destroyed[8].

High level of neutrophil elastase in saliva can be associated with the loss of active periodontal attachment. Moreover, neutrophil elastase can provide clear clinical symptoms of inflammation progress and diseases[7]. Neutrophil elastase is an indicator of neutrophil activities such as proteinase which is excreted from azurophilic granules. NE work optimally at pH 7 with zinc and magnesium as cofactors[4]. NE is inhibited by Serine Leukocyte Protease Inhibitor (SLPI) and α-antitrypsin which are found in the secretion of mucus and salivary gland. They affect the activities of NE in sub and supragingival. Epithelial cells in GCF is the main source of SLPI as an inhibitor[9].

The ability of tissues to stretch and support cells is obtained from elastin. Neutrophil elastase works as collagen is degrading collagen and elastin fibers [10, 11, 12]. The test of NE level is useful at the early stage of inflammation as neutrophil is the major cells that migrates in blood circulation to inflamed areas[9].

Macroscopically in oral cavity, periodontal disease is measured by gingival index, bleeding on proving, periodontal disease index and clinical attachment loss parameters. However, measurements done by using naked eyes parameters tend to create different perspectives between one operator and the other. Hence, the usage of physiology of body fluids that can depict the inflammation severity level, as well as tooth and periodontal tissues conditions accurately. In this research, GCF was used as the physiology of body fluid as it is retrieved around the gum gaps around the inflamed gingiva, with neutrophil elastase as one of the parameters in GCF. Therefore, this study aims to observe the relationship between neutrophil elastase and periodontal tissue damages at gingivitis and periodontitis.

MATERIAL AND METHOD

The research type used in this study was Cross Sectional Comparative Study where dependent and independent variables are analyzed at the same time. The study was conducted in Padang City Hospital, West Sumatra. There were three sample groups with age between 19 and 30 years old. They are healthy patients and patients with mild gingivitis and periodontitis. Total sample was 66 people with 22 people in each group. Sample was taken following consecutive sampling technique based on the exclusion and inclusion criteria. Exclusion criteria is sample who has consumed antibiotic and anti-inflammation for the last 3 months, smoking, pregnant, on menstruation cycle, having systemic abnormalities like diabetes mellitus, and having periodontal treatment for the last 3 months.

The first periodontal tissue examination used Periodontal Disease Index according to Russel. Gingival crevicular fluid was collected by using an absorbent paper and stored at -20˚C. The reagent used was elastase, human, kit, HK319-02, product assays, quantity 2x96 seconds, standard range 0.4 – 25 ng/ml, detection 0.4 ng/ml and working volume of 100 µl/well.

The time when GCF is retrieved should be paid attention to as there is Cicardian Periodicity of GCF, which means that there is a gradual increase of total GCF at 6 a.m. to 10 p.m. and it is decreasing afterwards.

Gingival crevicular fluid was collected at 08.00 a.m. GMT +7 following circadian periodicity. The area where GCF was extracted had to be cleaned from plaque. To equalize the condition and minimize the involvement of oral bacteria, the patients were instructed to gargle 2% chlorhexidine solution. Next, the lips were retracted and isolated by using cotton rolls. Then absorbent paper was inserted using a technique from superficial intracervical and left for 3 minutes and collected. Then it was put into an Eppendorf tube which had been filled with phosphate buffer solution. The specimen was labelled, and sample retrieved would be analyzed by ELISA and stored at -20˚C.

The data analysis was conducted using a statistic program. The total of Neutrophil Elastase was tabulated and verified statistically by using Kolmogorov Smirnov Test to test the normal distribution of data. Then the relationship between neutrophil elastase and periodontal disease index was analyzed by using ANOVA test.

According to Russel, Periodontal Disease Index test was done by using 6 tooth regions measurement criteria (16, 21, 24, 36, 41 and 44). Should there is one missing tooth from these 6 regions, and then it can be substitute by the adjacent tooth.

RESULTS

Samples were 65% female and 35% male. Each group consists of healthy, mild gingivitis and mild periodontitis samples. The average age of female is lower than the male, such as 21.57 ± 4.19 versus 24.13 ± 2.18. This difference is statistically insignificant in which the ages between male and female groups are equal (Table 1).

Table 1. Average age of research subjects based on gender

Age	Gender	n	%	Average	SD	p
	Male	23	35%	24.13	2.18	0.13
	Female	43	65%	21.57	4.19	

There is a difference of neutrophil elastase average level based on PDI group. The highest level was obtained at mild periodontitis with average of 9.38 ± 1.06 ng/dl and it is significant. The table below shows that mild gingivitis patients are 2.3 times at risk of NE level elevation compared to healthy patients. Whereas at mild periodontitis case, the risk is higher by 3.5 times than the risk at healthy condition (Table 2).

Table 2. The difference of neutrophil elastase averages level (ng/dl) in gingival crevicular fluid with periodontal disease based on PDI

Enzyme Type	PDI	f	Average	Standard deviation	p
Elastase	Healthy	22	2.65	1.28	0.00
	Mild Gingivitis	22	6.27	1.13	
	Mild Periodontitis	22	9.38	1.06	
	Total	66	6.01	3.47	

In order to see the differences between the groups, the study was proceeded with Post-Hoc Bonferroni test. Significant differences of neutrophil elastase average level between healthy – mild gingivitis and healthy – mild periodontitis as well as mild gingivitis – mild periodontitis groups were obtained ($p < 0.05$) (Table 3).

Table 3. The result of Post-Hoc Bonferroni test for neutronphil elastase enzyme level between PDI group

	NE Level		
PDI	Health	Mild Gingivitis	Mild Periodontitis
Healthy	-	0.000	0.000
Mild Gingivitis	0.000	-	0.000
Mild Periodontitis	0.000	0.000	-

DISCUSSION

This study shows that there is a meaningful relationship between neutrophil elastase level in gingival crevicular fluid and periodontal diseases ($p < 0.05$). 44 samples tested were observed to have an increase in neutrophil elastase level in inflamed areas compared to healthy subjects. This condition indicates that neutrophil elastase level in GCF is a risk factor for periodontitis as well as the progressive rise of alveolar bone loss[13].

NE activities are significantly related with the depth of probing. NE level increases with the severity of inflammation. There is a relationship between NE level and the depth of the pocket, NE level will increase as the periodontal pocket gets deeper. Averagely NE level is based on PDI groups in which mild gingivitis has 2.3 times increase from the healthy groups, whereas mild periodontitis increases by 3.5 times. The rise of neutrophil elastase in GCF aligns with the severity of periodontal diseases. There are meaningful relationships between neutrophil elastase (NE) level and tissue damages in periodontal cases.

Periodontitis is a chronic inflammation reaction which is triggered from pathogenic microorganisms. In a severe periodontitis, ulcerated epithelium causes bacteria invasion. Therefore, many immune competent cells are recruited to lamina propria in sulcular epithelium as a response to inflammation[14].

Due to periodontal inflammation, there is an increase of enzymatic molecules which describe the severity of diseases. Periodontal diseases index diagnosis is based on the measurement scale from biomarker that is useful for an accurate diagnosis and a successful measurement of periodontal disease therapy. The measurement of this enzyme shows whether there are periodontal pathogens, gingiva and periodontal inflammations, host-immune inflammation response to pathogen species and periodontal tissue damages. Gingival crevicular fluid (GCF) was selected as the biological medium. It has been used to detect or diagnose active diseases and predict patients with risks of periodontal diseases[15,24]. GCF consists of enzymatic and non-enzymatic compositions. The enzymatic components come from the host or bacteria. One of the components detected is neutrophil elastase (NE)[9,24].

Neutrophil elastase is in the main group of superfamily chymotrypsin serine protease which is excreted from as much as 3pg per cells from azurophilic granules. In healthy periodontal tissues, this protein is surrounded by collagen fibres to protect it from collagenases activities[24]. This enzyme is also found in a small amount in monocytes and mast cells. NE can degrade extracellular matrix proteins including elastin, collagen (type I – IV), fibronectin, laminin and proteoglycans. Moreover, it can break coagulation factors (fibrinogen and factors V, VII, XII, XIII), plasminogen, IgG, IgA and IgM, C3 and C5 complement and receptor factors, antigen leukocyte, thrombin receptors and gp120 also proteins of HIV. NE also breaks another protease in neutrophil granules[8]. It induces the released of proinflammatory cytokine like IL-6, IL-8 and granulocyte macrophage colony stimulating factor (GM-CSF). Therefore it prolongs the inflammation process.

Tissue damages in periodontal diseases are mainly caused by hydrolase enzyme which is released by inflammation, fibroblast and epithelial cells followed by their activities. Many studied enzymes, like collagenase, gelatinase, elastase and β-glucuronidase, are indicators to periodontal health. Neutrophil elastase (NE) plays a role in tissue damages during periodontal inflammation. NE level in GCF and saliva is correlated with periodontal status, and then it will go back to normal level during recovery[16].

Neutrophil is a major cell that is migrated in blood circulation to inflamed areas. The increase of NE can be detected in periodontal ligament in chronic periodontal diseases than in healthy cases. Healthy periodontal ligament contains collagen fibres that are covered by 110 kDa acidic glycoprotein non collagenous fibres degraded by elastase. Among proteinase found in periodontal ligament (neutrophil elastase, plasminogen and metalloproteinase-9 matrix), only NE that can degrade non-collagenous protein in periodontium.

Ujiie et al (2008) study stated that NE causes the degradation of oxytalan fibres. It also supports blood flow and lymphatic to teeth and degrades inter - fibrillar substantiation in periodontal ligament that causes the weakening of mechanical strength in periodontal ligament to support the tooth[17]. NE degradation activity can be observed since the early lesion periodontitis hence NE level measurement can be beneficial and be done at that stage[18].

Neutrophil elastase is the most destructive enzyme in the body with its ability to degrade extracellular matrix proteins (including collagen, proteoglycan, fibronectin, platelet and complement receptors, thrombomodulin, pulmonary surfactant and cadherins) also plasma proteins (including complement actor, immunoglobulin, several proteases and the inhibitor). In physiology condition, protease inhibitor, like alpa1-antitrypsin, can regulate NE active ties[4].

In physiology state, antiprotease (NE inhibitor, α-1 antitrypsin) prevents NE destructive effect because the level is higher than NE. Hence "antiprotease screen" is formed. NE concentration in on neutrophil is more than 5mM. High elastase concentration is regulated by the division of compartments in azurophilic granules. Antiprotease enzyme like α anti-trypsin and SLPI arrange NE proteolytic activities secreted by the neutrophil. Neutrophil has got a short lifespan, as much as 250 mg of NE must be eliminated each day, which is why the total of NE and its inhibitors must be balanced[8].

In pathological condition, antiprotease is degraded by the oxide compounds from bacteria phagocytosis like O_2^-, H_2O_2, OH and OCl^-. Consequently, NE concentration is higher than the inhibitor and results in the damages of tissues[19]. Pro-inflammatory cytokines such as interleukin (IL)-6 and IL-8 activate neutrophil functions and migration to inflamed tissues. With the increase of neutrophil in inflamed tissues, NE increases and damages occur in the tissues[20].

In periodontal diseases, inflammation is caused by bacteria invasion which causes neutrophil phagocytosis of microbes then following apoptosis process which is known as "phagocytosis-induced cell death". Such process in benefiting the host as neutrophil elimination that consists of microbes took place. At several pathogenic bacteria like P. Gingivalis, such process can be inhibited. The pathogen induces cells to produce IL-8 to attract more neutrophil to migrate to inflamed periodontal tissue areas. As a result NE level increases, inflammation is prolonged and tissues are damaged[25,26]. The destruction of elastin is caused by the increase of chemotaxis, immune cell proliferation and gingiva degradation in epithelium cells due to the rise of neutrophil elastase level activated by IL-8, IL-6 and PGE[15,21]. The study done by Geraghty stated that the tissue location with high elastase level indicated the progressing increase to the risk of alveolar bone loss.

Neutrophil elastase also causes the damages in alveolar bones. This process occurs due to the mecha nism from Del-1 and IL-17. The two molecules act as inhibitors to each other. Del-1 is as one of the inhibitors to neutrophil; this molecule inhibits the migration of neutrophil as well as IL-17 production. Vice versa, IL-17 inhibits Del-1 expression and stimulates neutrophil infiltration. In case of chronic inflammation, IL-17 sends a signal which causes the continuous migration from neutrophil elastase. The high IL-17 will stimulate the expression of RANKL and induce resorption process[27].

The important bone resorption component is albumin matrix degradation which is influences by proteolytic enzyme that work at optimum pH. In alveolar bones atrophy, the most destructive enzyme is NE at pH 6.5[22]. According to Loss (2000), the measurement of NE at early stage of inflammation is very advantageous as neutrophil migrates in the blood to inflamed locations[9,23].

It can be concluded that neutrophil elastase level in gingival crevicular fluid is related to the level of damage in tissues at gingivitis and periodontitis. NE usage onwards can be recommended as one of the parameters to measure periodontal diseases.

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