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Correlation Between Matrix Metalloproteinase 8 in Gingival Crevicular Fluid and Zinc Consumption

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Abstract: Gingivitis is a disease affecting the gingival tissues, when the gingivitis is not properly treated, it will lead to a destructive of periodontal tissue structure. Periodontitis can cause periodontal tissue destruction and loss of the dental arch, further facial abnormalities and gastrointestinal disorder. Zinc is Matrix Metalloproteinase-8 cofactor. This study aims to prove the relationship between levels of Matrix Metalloproteinase-8 of the Gingival Crevicular Fluid (GCF) and zinc consumption of the Minangkabaunese in West Sumatera, Indonesia. This study involved 60 subjects, consisted of 20 healthy subjects, 20 patients of mild gingivitis and 20 patients of mild periodontitis. Matrix Metalloproteinase-8 level was tested by using ELISA method. Consumption of zinc was measured by using Minangkabau Food Frequency Questionnaire (FFQ). Data analysis was performed by means of univariate to describe each variable. Kolmogorov Smirnov Test was used to analyze normal distribution. Pearson correlation was applied to correlate between Matrix Metalloproteinase-8 level and zinc consumption. This study concluded that there was a significant correlation between the levels of matrix metalloproteinase-8 and zinc consumption. Relationship between Matrix Metalloproteinase-8 and zinc consumption showed a strong correlation with negative direction ($r = -0.865$). Supplementation of zinc maybe used as a treatment for periodontal inflammation.

Key words: Matrix Metalloproteinase-8, zinc, periodontal disease

INTRODUCTION

Periodontal disease is a very common inflammatory disease. Bacteria are the initiating factors, periodontitis is not a classical infectious disease. Majority of the causative organism are also present in the healthy mouth (Socransky and Haffajee, 2005).

In periodontal disease, Matrix Metalloproteinase-8 level increases. Matrix Metalloproteinase-8 (MMP-8) is a Matrix Metalloproteinase (MMPs) group. MMPs is secreted in latent form. By process transcription, zymogen precursor, MMPs becoming MMP-8, MMP 1 and MMP 13. MMP-8 inhibitor is TIMPs (Tissue Inhibitor Matrix Metaloproteinase) (Charoenrat *et al.*, 2001; Amaleinei *et al.*, 2007).

In neutral pH, MMP 8 is activated when degraded by extracellular protein. MMP-8 is stored on neutrophil secondary granule which is secreted by autolytic. MMP-8 activated by protease which works at pH 7 and optimum temperature (37°C) (Nagase *et al.*, 2006).

The most collagen in gingival fibers is type I collagen. MMP-8 degrades type I collagen which is a major part of gingival fibers. The function of type I collagen are tighten firmly against the gingival edge of the tooth, provide the necessary rigidity to resist the forces of mastication without the need to avoid tooth surfaces, uniting the free marginal gingiva and the root cementum of the adjacent attached gingiva (Fehren Bach and Weiner, 2009).

Type I collagen continuous destruction will increase MMP-8 production and gingivitis will turn into periodontitis. In the state of mild gingivitis, it is painless so it makes less attention, but if it didn't get a proper treatment can turn into periodontitis, a destructive form of periodontal tissue that cause the loss of periodontal tissue structure and loss of the dental arch and facial abnormalities and gastrointestinal disorder. On the condition of periodontitis, there is high number of anaerobic bacteria in the periodontal supporting tissues, which is a local infection in the body tissues and other organs that will infect systemically through the blood vessels and can lead to death (Perinetti *et al.*, 2008). Matrix Metalloproteinase-8 is family of calcium-dependent zinc-containing endopeptidases, which are responsible for the tissue remodeling and degradation of the extracellular matrix (ECM). Zinc has important role in MMP-8 reaction (Verma and Hansch, 2007). The reaction mechanism for the proteolysis by MMPs has been delineated on the basis of structural information. It is proposed that the scissile amide carbonyl coordinates to the active-site zinc (II) ion. This carbonyl is attacked by a water molecule, which is both hydrogen bonded to a conserved glutamic acid (Glu-198 in MMP-8) and coordinated to the zinc (II) ion. The water molecule donates a proton to the Glu residue that transfers it to the nitrogen of the scissile amide, which is followed by

the Glu residue shuttling the remaining proton from the water molecule to the nitrogen of the scissile amide with resultant peptide bond cleavage. In this process, the positively charged zinc (II) ion helps to stabilize a negative charge at the carbon of the scissile amide and a conserved alanine (Ala-161 in MMP-8) residue helps stabilize positive charge at the nitrogen of the scissile amide (Whittaker *et al.*, 1999).

Increasing number of bacteria in mouth will enhance level of MMP-8 in inflammation process. MMP-8 as Matrix metalloproteinase-8 (MMP-8) or collagenase-2 is one of central biomarkers in the connective tissue in periodontal disease (Sorsa *et al.*, 2006). Cellular response is characterized by the presence of neutrophils which are hallmark of acute inflammatory reaction. Because of increase in vascular permeability PMN's emigrate from blood vessels into the connective tissue by a process called trans endothelial migration to Gingival Crevicular Fluid (GCF) and saliva (Zaverio and Shaun, 2002).

Zinc affects multiple aspects of the immune system. Zinc is essential for normal development and function of cell-mediating innate immunity, neutrophils and natural killer cell (Overbeck *et al.*, 2008). Zinc plays an important role as oxidative stress and also an anti-inflammatory agent. These unique properties of zinc may have significant therapeutic benefits in several disease in human. In many disease, such as periodontal disease, concurrent zinc deficiency may complicate the clinical features, affect adversely immunological status, increase oxidative stress and increase the generation of inflammatory cytokines (Prasad, 2008).

This study aims to prove the relationship between levels of Matrix Metalloproteinase-8 of the Gingival Crevicular Fluid (GCF) and zinc consumption of the Minangkabau in West Sumatera, Indonesia.

MATERIALS AND METHODS

Sixty patients aged between 17-30 years from Dental Clinic of Rasyidin Municipal Hospital Padang in West Sumatera, Indonesia was recruited. The sample consisted of ethnic Minangkabau who consume Minangkabau food daily. Subjects who consume antibiotics and antiinflammatory during the last 3 months, smokers, pregnant, menstruation, systemic disorders such as diabetes melitus and got a history of periodontal treatment during the last 3 months were excluded.

Patients were categorized into three groups, mild gingivitis, mild periodontitis and healthy control group. Mild gingivitis was characterized by reddish gingiva, oedema, shiny and bleeding on palpation. Mild periodontitis characteristic is gingival sulcus which was measured on one of the two sides, extends 3 mm gingival sulcus from apical of Cemento Enamel Junction (CEJ). Healthy control group showed no signs of inflammation, no bleeding and no attachment loss, with

coral pink colored gums. Informed consent was obtained from all patients. Ethical clearance was approved from the Ethics Committee of Faculty of Medicine of Andalas University (No 247/KEP/FK/2013). In collecting the GCF samples, subjects were instructed to sit in the dental unit with ergonomic position and to rinse with a solution of 2% Chlorhexidin, to equalize conditions and minimize the involvement of oral bacteria. Plaque was removed in the area to be taken GCF and blew with three way syringe. Lip retractor was paired. Then GCF lection was isolated with cotton role. Paper points were inserted by using the technique of superficial intra crevicular then left for 3 min. Paper points were taken and put in eppendorf tubes which is already containing Phosphate Buffer Solution (PBS). Specimens were labeled clearly. Samples which were taken to be analyzed by ELISA were stored at -20°C until sufficient number of samples according to the instructions contained in the kit.

The measurement of MMP-8 in GCF which collected from gingival pocket from three groups was tested using ELISA sandwich method. ELISA kit using Reagan KIT MMP-8 RPN2619, Human Biotrak System by Amersham Biosciences. After termination of the ELISA reaction, ELISA reader immediately carried out with maximum wavelength of 450 nm for 30 min. The number coming out of the ELISA Reader was absorbent figures which should be compared with the help of standard chart was made after examination by ELISA Reader, the turn into numbers of MMP-8. Laboratory test performed at the Biomedical laboratory of Faculty of Medicine of Andalas University and worked with the quality assurance and resource who were competent in their field. The tools were calibrated according to standard laboratory procedures on a periodic basis by the Quality Control System, to determine the price of coefficient of variation (CV).

The assessment of zinc consumption was done by asking all participants to answer a food frequency questionnaire consist of 220 Minangkabau food with various cook methods such as curry, frying, boiling, grilling and stir-frying.

MMP-8 and zinc level were tabulated and tested statistically using the Kolmogorov Smirnof Test to determine normal distribution of data, then correlation of MMP-8 and zinc was tested using Pearson correlation test.

RESULTS

Samples were taken from patients who visited the hospital dental clinic of Rasidin Municipal Hospital Padang from June to December 2013. The results of the examination of 200 prospective subjects from 1200 visitors to the hospital dental clinic were collected 60 subjects consisted of 20 healthy subjects, 20 patients with mild gingivitis and 20 patients with mild periodontitis.

Table 1: Difference of matrix metalloproteinase-8 levels (ng/dl) in gingival crevicular fluid with periodontal disease based on periodontal disease index (PDI)

Enzyme	PDI	f	Mean	SD	p
MMP8	Healthy	20	4.21	4.41	0.00
	Mild gingivitis	20	21.69	3.94	
	Mild periodontitis	20	40.16	2.64	
	Total	60	22.02	15.25	

Table 2: Difference of zinc consumption (mg) from food frequency Questionnaire in 220 kinds of Minangkabaunese food based on PDI

	PDI	F	Mean	SD	p
Zinc	Healthy	20	7.09	1.12	0.00
	Mild Gingivitis	20	5.25	0.50	
	Mild Periodontitis	20	3.78	0.54	
	Total	60	5.37	1.57	

Table 3: Correlation analysis of MMP-8 and zinc consumption of minangkabaunese food

Level of MMP-8	Level of Zinc	R	p
		-0.865	0.000

Table 1 showed significant difference in the levels of Matrix Metalloproteinase-8 on the terms of the PDI group, the highest MMP-8 was shown by mild periodontitis group with mean = 40.16±2.64 ng/dL. The table above showed that the mild gingivitis patients likely to have elevated levels of MMP-8 5.2-fold compared to healthy condition, while the condition of mild periodontitis rose 9.5-fold compared to healthy conditions.

Table 2 showed significant difference in the levels of zinc consumption on the terms of the PDI group, the highest zinc consumption was shown by healthy control with mean = 7.09±1.12 mg. The table showed that the mild gingivitis patients likely to have zinc consumption with mean = 5.25±0.50 mg. In mild periodontitis condition with mean = 3.78±0.54 mg.

Statistic analyze by using Pearson correlation test showed that coefficient of Pearson correlation (r) is -0.865 with significance level p = 0.000 between Matrix Metalloproteinase-8 enzyme and zinc consumption.

DISCUSSION

Periodontal disease occurs due to the interaction of bacteria with host antigen-tissue that activates neutrophils, antibody production and bone resorption (Nagase *et al.*, 2006). Neutrophil function to control both phagocytosis and bacterial attacks also issued a Matrix Metalloprotease-8 (MMP-8), which could contribute to tissue damage (Cox and Overall, 2008).

Matrix Metalloproteinase-8 enzyme has an important role in tissue destruction during inflammatory processes (Apajalahti, 2004). Matrix Metalloproteinase-8 is also important in metabolism of peptidoglycan in the cell wall of gram positive and gram negative bacteria that activates complement, immunosuppressive activity, stimulates bone resorption and stimulates macrophages to produce prostaglandin and collagenase (Chung, 2004).

Increased levels of Matrix Metalloproteinase-8 started in mild gingivitis, subsequently became chronic, occurring periodontitis is characterized by the destruction of alveolar bone and even periodontal ligament. In a change from healthy periodontium be inflammatory, collagen breakdown occurred change of intracellular pathways to the controlled Metalloproteinase-mediated pathway (Kinane *et al.*, 2003).

Matrix Metalloproteinase-8 levels were also detected in a healthy and correlated with periodontal health status. The higher levels of MMP-8 in gingival sulcus, the higher the degree of periodontal destruction. Local levels of MMP-8 in the gingival sulcus of healthy control of inflammation associated with activity against microbes (Norppa, 2012).

This research result showed a significant difference in the levels of Matrix Metalloproteinase-8 on the terms of the PDI group, the highest MMP-8 was shown by mild periodontitis group with mean = 40.16±2.64 ng/dL. The highest zinc consumption was shown by healthy control with mean = 7.09±1.12 mg. The table showed that the mild gingivitis patients likely to have zinc consumption with mean = 5.25±0.50 mg. In mild periodontitis condition with mean = 3.78±0.54 mg. Zinc is essential for normal development and function of cell-mediating innate immunity, neutrophils and natural killer cell (Overbeck *et al.*, 2008). Zinc plays an important role as oxidative stress and also an anti-inflammatory agent. These unique properties of zinc may have significant therapeutic benefits in several disease in human. In many disease, such as periodontal disease, concurrent zinc deficiency may complicate the clinical features, affect adversely immunological status, increase oxidative stress and increase the generation of inflammatory cytokines (Prasad, 2008). Gingival Crevicular Fluid (GCF) collection describes amount of MMP-8 based on inflammation level in periodontal disease. Examination FFQ in Minangkabaunese diet showed zinc supplements would decrease the expression of Matrix Metalloproinase-8 that were supposed to impair wound healing in case of an over-expression.

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