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Research Report

Correlation between magnesium and alkaline phosphatase from gingival crevicular fluid on periodontal diseases

Nila Kasuma
Department of Oral Biology
Faculty of Dentistry, Universitas Andalas
Padang– Indonesia

ABSTRACT

Background: *Magnesium is one of the alkaline phosphatase (ALP) cofactor. The amount of magnesium contained infoods affect ALP activity. Increased ALP activity will indicate the level of inflammation in periodontal disease. Elevated inflammation in periodontal disease will change gingivitis to periodontitis, where there has been damage to the bone ssupporting the teeth, and an increasing number of gingival crevicular fluid (GCF). The content of GCF consists of enzymatic and non-enzymatic. Changes in the composition of GCF occurs when the inflammation gets worse.* ***Purpose:*** *This study was aimed to prove the correlation between magnesium and ALP from GCF on periodontal disease.* ***Method:*** *This research involved 60 Minangkabaunese people with 20 healthy samples, 20 mild gingivitis samples, and 20 mild periodontitis samples. GCF was collected by absorbing method. Then ALP level in GCF was measured by using ELISA technique. Magnesium level in Minangkabaunese food was tested by Food Frequency Questionnaire (FFQ).Univariate analysis was performed to describe each variable. To see a normal distribution, Kolmogorov Smirnof Test was used (p>0.05). Unpaired T-test and Pearson correlation test was used to see correlation between ALP and magnesium level in Minangkabaunese food.* ***Result:*** *There is a significant correlation between the levels of ALP and magnesium level in Minangkabaunese food with periodontal disease (p=0.005). ALP is higest on mild periodontitis (137.74±23.01 ng/dl). Magnesium level normal control group is highest (250.14±32.34 mg) and in mild periodontitis is the lowest (110.83±21.04 mg). Corelation between ALP and magnesium level indicates strong correlation with negative direction (r=- 0.907).* ***Conclusion:*** *There is correlation between the levels of alkaline phosphatase and magnesium level on periodontal disease. Increasing inflamation rate will elevate the ALP level.*

Keywords: *alkaline phosphatase; magnesium; periodontal disease*

Correspondence: Kasuma N, c/o: Departemen Biologi Oral; Fakultas Kedokteran Gigi Universitas Andalas. Jln. Perintis Kemerdekaan No. 77 Padang, Indonesia. E-mail: nilakasuma10@gmail.com

INTRODUCTION

Alkaline phosphatase (ALP) is produced by many cells. ALP is the major source of polymorphonuclear (PMN), bacteria in supragingival plaque and subgingival and activity of osteoblasts and fibroblasts, and a small contribution to the serum.[1] ALP predominantly found in the pocket epithelium. If the main source of ALP in periodontal disease is PMN, the ALP is a potential marker for inflammation.[2]

Increased accumulation of plaque and inflammation will improve periodontal tissues pathological inflammation. It triggers bacterial endotoxin to activate prostaglandins , which in can activate osteoclasts . In this condition B cells are stimulated to produce IL-1b and TNFa to destroy bone, then it causes bone loss. Increased activity in periodontal disease is due to increased inflammation and bone turnover rate. Periodontal disease progress will be equal with ALP activation. Due to the increased severity of the inflammation, bone turnover rate elevates.[3,4]

One of ALP cofactor is magnesium. Most studies[5] have agreed on the correlation of magnesium and ALP activity. The results show that assays of ALP activity in homogenised tissue samples will give better responses if both $Mg^{2+}$and

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Zn^{2+} ions are included in the reactions. The purpose of this study is to see the relationship between magnesium level and concentration of ALP in gingival crevicular fluid on normal, mild gingivitis and periodontitis group.

MATERIALS AND METHODS

This study is a cross sectional comparative study, dependent and independent variable is examined on the same time to see the magnesium level in 3 groups of sample. Samples were taken by consecutive sampling technique based on exclusion and inclusion criteria. Population is patients who seek for treatment in Dental Polyclinic of RSUD Padang–West Sumatera–Indonesia.The sample consisted of ethnic Minangkabaunese who consume Minangkabau food daily.Ages ranged between 17-30 years. To minimalize the local factor which can influence the activity of ALP, at the time of examination , patients are systemically were sistemically healthy and didn't consume antibiotics during the last 3 month. Patients also didnt have any teeth caries. If samples consume antibiotics and antiinflamatory during the last 3 months, have any teeth caries, smokers, pregnant, menstruation, have a systemic disorders such as diabetes melitus,and got a history of periodontal treatment during the last 3 months are excluded from this research. Informed consent was obtained from all patients.

The first examination of periodontal tissues using the Periodontal Disease Index (PDI) according to Ramfjord.[6] Examination using the instrument a periodontal probe. This tool is used to measure the depth of pockets. Normal depth of the pockets around 0-3 mm. Scores PDI are: 0=healthy gingiva, absence of signs of inflammation no bleeding and no attachment loss, with coral pink colored gums; 1=mild to moderate inflammatory changes not extending around the tooth; 2=mild to moderately severe gingivitis extending all around the tooth; 3=severe gingivitis characterized by marked redness, swelling, tendency to bleed and ulceration; 4=mild periodontitis, assigned if the loss of attachment is 3 mm or less; 5=is assigned if the loss of attachment is greater than 3 mm but less than 6 mm; 6=if the loss of attachment is 6 mm or more, a score of 6 is given to a particular tooth.

Six measurement criteria teeth region (16, 21, 24, 36, 41 and 44) on mesial, bucal, distal, and lingual site represent periodontal disease indexes. If a ramfjord tooth was missing, a substitute tooth which was selected is teeth numbers 17, 11, 25, 37, 31, 45. In this study only 3 groups (healthy control, mild gingivitis, and mild periodontitis) were taken. Selection of 3 groups of PDI is to examine changes on healthy, mild gingivitis, mild periodontitis group. Clinical charactheristic of mild gingivitis are erythema and minimal bleeding on probing, the same as the characteristic of stage II of gingivitis, the early lession. Comparison of ALP level on the three groups illustrates the increase of ALP level is detectable on mild gingivitis and mild periodontitis state. According to Sanikop, ALP level increases in mild gingivitis due to tissue alteration as a result of host-parasite reaction or host-bacterial interplay as gingivitis is an inflammatory process. During progression of the disease to mild periodontitis, enzymes are released from dead and dying cells of the periodontium, PMNs, inflammatory, epithelial, and connective tissue cells of the affected sites, so the level in mild periodontitis is highest among the three groups.[1] Increased ALP level in early inflammatory can expect the early detection of periodontal disease. Each group consisted of 20 persons.

Gingival crevicular fluid (GCF) was collected using absorbent paper (2.55 mm wide, 0.16 mm thick, and 14.19 mm long; 8.07 mm of the length was identified as a handling area with a blue tape).[8] GCF sample was stored at -20C. Reagent use is Elisa Kit for ALP,[9] homosapiens (Human), sE91472Hu with detection range 3.12-200 ng/ml and sensitivity1.36 ng/ml, USCN product by spectrophotometer variant hemoglobin testing-in2it analyzer – Bio rad. The GCF was diluted 20 times, 10µL is added to 190µL RD5-10 Calibrator diluent. GCF then diluted 40 times that 10µL sample was added to 390µL calibrator diluent. Diluted GCF is taken 50 mL and then was diluted 7 times with 150 mL of normal saline, to the make 200µL of sample. The diluted GCF was then centrifuged for 3–5 minutes at 3500–4500 rotations per minute (rpm) in a microcentrifuge; 140µL of the clear supernatant was utilized for the analysis of ALP level.The enzyme level was recorded using ELISA readerat a wavelength of 450 nm.

GCF was collected in the morning at 08.00 am according to circardian periodicity. Collecting area was minimalized of plaque. To equalize the conditions and minimize the involvement of oral bacteria instructed the patient to rinse using a solution of 2% chlorhexidine. Then the lips retracted and isolated using cotton roll. Absorbent paper is inserted by using the technique of superficial intracrevicular and left for 30 seconds.[10] Sample which is contaminated by blood will not be taken. Absorbent paper is taken and placed in Eppendorf tube that has been filled with 1 ml phosphate buffer solution. Specimens are labeled. The samples taken is stored at -20 C and be analyzed using ELISA. Then participant answered a food frequency questionnaire consist of Minangkabau food. Before answering questionnaire, participant is shown 220 kinds food dummies to help remembering food they consume daily. Portion, kind, and size of food has been determined. In this study, we use foods which contain magnesium. On the FFQ interview, sample selected dummy food. This is useful for reducing bias in answering this questionaire. In this study, the relationship between magnesium level in food and ALP level is tested at the same time. Sample was divided into three groups of healthy , mild gingivitis and mild periodontitis. Then GCF is collected to measure ALP level. Patients were interviewed by a professional nutritionist using FFQ method which is equipped with a dummy table of all 220 kinds of food. Magnesium level measurement is carried out from FFQ in

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220 kinds of Minangkabaunese food with way of cook like curry, frying, boiling, grilling, and stir-frying.

ALP and magnesium level were tabulated and tested statistically using the Kolmogorov Smirnof Test to determine normal distribution of data. Pearson correlation test was used to see then correlation of ALP and magnesium.

RESULTS

Samples were taken from patients who visited the hospital dental clinic Rasidin Municipal Padang for 4 months from June to December 2014. The results of the examination of 200 prospective research subjects than 1200 visitors to the hospital dental clinic Rasidin Municipal Padang, has netted 60 subjects consisted of 20 healthy subjects, 20 subjects with mild gingivitis and 20 subjects with mild periodontitis Minangkabaunese foods and met the inclusion criteria through consecutive sampling method.

Based on Table 1 there is significant difference in the levels of ALP on the terms of the PDI group, which is highest in the mild periodontitis with mean=137.74± 23.01ng/dl. The table above shows the mild gingivitis patients likely to have elevated levels of ALP 3,6 -fold compared to healthy condition, while the condition of mild peridontitis rose 5,3-fold compared to healthy conditions.

Based on Table 2, there is significant difference in the levels of magnesium level on the terms of the PDI group, which is lowest in the mild periodontitis with mean=110.83 ±21.04 mg. The table shows the mild gingivitis patients likely to have elevated levels with mean=164±18.31 mg. In healthy condition with mean=250.14±22.34 mg. Statistic analyze by using Pearson correlation test shows that coefficient of Pearson correlation (r) is -0.907 with significance level (p) 0.000 (p<0.05) between ALP enzyme and magnesium.

DISCUSSION

The results of statistical tests in this study found a significant difference between the levels of ALP in GCF with periodontal disease (p<0.05). ALP in GCF was significantly higher in periodontitis compared to healthy samples and gingivitis.

Amongst the host enzymes in GCF, ALP was one of the first to be identified. ALP is a membrane bound glycoprotein produced by many cells within the area of periodontium and gingival crevice. It is released from polymorphonuclear neutrophils during inflamation, osteoblast during bone formation, periodontal ligament fibroblast during periodontal regeneration.[7] ALP activity is valuable to clinicians because enzymatic modification occure at the GCF level earlier than clinically evident modification.

ALP is synthesized and secreted by osteoblasts during the period of phenotypic maturation of osteoblasts, three consecutive phases of proliferation, extracellular matrix maturation and mineralization in bone tissue.[11] Thus it makes sense that osteoblasts synthesize and secrete alkaline phosphates creating a local bone environment of alkalinity by splitting of phosphorus (an acidic mineral) creating an alkaline pH to help bone mineralization. ALP is normally presents in high concentration in growing bones and it is also known as zinc - dependent enzyme. Increased activity in periodontal disease will equal with ALP activation. Due to the increased severity of the inflammation, bone turnover rate elevates.[4,2,12] Bone turnover is a process in which the circulation of bone osteoclasts break down bone, and osteoblasts work rebuilding bone. In severe periodontal disease, the increased bone turnover intensifies work of destroying osteoclasts in bone.[13-15]

ALP is an enzyme the which is synthesized by the liver, bone, and less amount by the intestines and kidney. As the name implies, this enzyme works best at an alkaline

Table 1. Alkaline Phosphatase levels (ng/dl) in gingival crevicular fluid on healthy, mild gingivitis and mild periodontitis group

Enzyme	PDI	F	Mean	SD	p
ALP	Healthy (score 0)	20	25.78	19.69	0.00
	Mild to moderate inflammation on gingiva (score 1)	20	92.92	19.23	
	Mild periodontitis (score 4)	20	137.74	23.01	
	Total	60	85.48	61.93	

Table 2. Magnesium levels (mg) from FFQ on healthy, mild gingivitis and mild periodontitis group

	PDI	F	Mean	SD	p
Magnesium	Healthy (score 0)	20	250.14	22.34	0.00
	Mild to moderate inflammation on gingiva (score 1)	20	164	18.31	
	Mild Periodontitis (score 4)	20	110.83	21.04	
	Total	60	174.99	61.69	

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Table 3. Correlation analysis of ALP and magnesium level in Minangkabaunese food

Level of ALP	Level of Magnesium	r	*p*
		-0.907	0.000

pH (pHš10), and thus the enzyme itself is inactive in the blood. ALP removes phosphate groups (known as dephosphorylation) from many types of molecules, including nucleotides, proteins, and alkaloids.[1,2] ALP contains two magnesium ion (Mg^{2+}) and four zinc ion (Zn^{+}). Thus, these metal ions are required in defined optimal ratio. Magnesium ion is usually employed as the only cofactor of ALP. The ALP from various mammalian tissues are among the enzymes known to be activated by magnesium in isolated systems.[5]

In this study, magnesium level is the lowest on mild periodontitis, and highest on healthy control group. Meisel *et al*. investigated the association between magnesium status and periodontal health in a population based analysis. Increased serum magnesium was significantly associated with reduced probing depth, attachment loss, and high number of remaining teeth. A decrease of magnesium will be followed by a decrease in ALP activity. Long-term loss of magnesium from the bone causes disturbances of bone modeling, remodeling, and turnover, with resultant bone abnormalities.Nutritional magnesium supplementation may improve periodontal health.[16]

Magnesium is the fourth most abundant mineral and the second most abundant intracellular divalent cation and has been recognized as a cofactor for >300 metabolic reactions in the body. Some of the processes in which magnesium is a cofactor include, but are not limited to, protein synthesis, cellular energy production and storage, reproduction, DNA and RNA synthesis, and stabilizing mitochondrial membranes. Magnesium also plays a critical role in nerve transmission, cardiac excitability, neuromuscular conduction, muscular contraction, vasomotor tone, blood pressure, and glucose and insulin metabolism.[17] Because of magnesium's many functions within the body, it plays a major role in disease prevention and overall health. Low levels of magnesium have been associated with a number of chronic diseases including migraine headaches, Alzheimer's disease, cerebrovascular accident (stroke), hypertension, cardiovascular disease, and type 2 diabetes mellitus. Good food sources of magnesium include unrefined (whole) grains, spinach, nuts, legumes, and white potatoes (tubers). This review presents recent research in the areas of magnesium and chronic disease, with the goal of emphasizing magnesium's role in disease prevention and overall health.[18] The body of most animals contains ~0.4 g magnesium/kg.[14] The total magnesium content of the human body is reported to be ~20 mmol/kg of fat-free tissue. In other words, total magnesium in the average 70 kg adult with 20% (w/w) fat is ~1000 to 1120 mmol or ~24 g. About 99% of total body magnesium is located in bone, muscles and non-muscular soft tissue.[19]

Magnesium concentration can affect the stimulation of phosphatase activity.[19] This suggests that magnesiummediates stabilization and destabilization of the catalytically active structure of ALP concentrations at low and high respectively. Magnesium is thought to have a regulatory effect on the expression of catalytic activity and maintenance of the structural integrity of the enzyme.[5] Magnesium alone does not activate the apoenzyme but it regulates the nature of the zinc - dependent restoration of catalytic activity to apophosphatase, increasing the activity of the enzyme containing 2 g - atoms of zinc five fold and that of enzyme containing 4 g atoms of zinc 1.4-fold. Hence magnesium, the which is specifically bound to the enzyme, both stabilizes the structure and dynamic protein regulates the expression of catalytic activity by zinc in alkaline phosphatase.[20] Magnesium acts as an activator within optimal concentrations but became inhibitory at higher concentration. One possible explanation for this result is that excess of magnesium ions displaced zinc ion from the catalytic site since both metal ion can bind to the same site. The higher magnesium intake the lower ALP activity will be.[5] Thus periodontal disease progression also decrease. Practitioner can also provide appropriate additional therapy by giving magnesium supplement to suppress the periodontal inflammation.[20] In conclusion, there is correlation between the levels of alkaline phosphatase and magnesium level on periodontal disease. Increasing inflamation rate will elevate the ALP level.

REFERENCES

1. Sanikop S, Patil S, Agrawal P. Gingival crevicular fluid alkaline phosphatase as a potential diagnostic marker of periodontal disease. J Indian Society of Periodontology 2012; 16(4): 513-8.
2. Malhotra R, Grover V, Kapoor A, Kapur R. Alkaline Phosphatase as a periodontal disease marker. Indian J Dent Res 2010; 21: 531-6.
3. Guerrera, MP, Stella LV, Jun JM. Therapeutic uses of magnesium. Am Fam Physician 2009; 80(2): 157-62.
4. Al-Rawi NA, Rashad JM. Salivary alkaline phosphatase and periodontal disease. J Bagh College Dentistry 2013; 25(1): 137-9.
5. lorunniji FJ, Adedoyin I, Joseph OA, Rotimi OA, Sylvia OM. Cofactor interactions in the activation of tissue non-specific alkaline phosphatase: synergistic effects of Zn2+ and Mg2+ Ions . BIOKEMISTRI 2007; 19(2): 43-8.
6. Ramfjord SP. The periodontal diesase index (PDI). J Periodontol 1967; 38: 602-10.
7. Carranza FA. The periodontal pocket. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA, eds. Clinical periodontology, 11th ed. St. Louis: Saunders-Elsevier; 2012. p. 434-51.
8. Silva FGA, Sabrina CG. Validation of an alternative absorbent paper for collecting gingival crevicular fluid. Periodontia 2009; 19(3): 85-90.
9. Kim HJ, Kwak J. Electrochemical determination of total alkaline phosphatase in human blood with a micropatterned ITO film. J Electroanalytical Chemistry 2005; 577(2): 243-8.
10. Guentsch A, Kramesberger M, Sroka A, Pfister W, Potempa J, Eick S. Comparison of gingival crevicular fluid sampling methods in patients with severe chronic periodontitis. J Periodontol 2011; 82(7): 1051-60.
11. Perinetti G, Paolantonio M, Femminella B, Serra E, Spoto G. Gingival crevicular fluid Alkaline Phosphatase activity reflects periodontal healing/recurrent inflammation phases in chronic Periodontitis patients. J Periodontol 2008; 79: 1200.

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DOI: 10.20473/j.djmkg.v48.i3.p130-134

12. Üsal B, Işıl S, Özlem D, Bal B, Bolu E. The relationship between periodontal status and alkaline phosphatase kadars in gingival crevicular fluid in men with hypergonadotropic hypogonadism. Yonsei Med J 2008; 49(1): 71–8.
13. Neve A, Addolorata C, Francesco PC.Osteoblast physiology in normal and pathological conditions. 2010. Cell Tissue Res.
14. Seibel MJ. Biochemical markers of bone turnover part I: biochemistry and variability. Clin Biochem Rev 2005; 26(4): 97–122.
15. Rani K, Sanchi D, Rachita R. Brief review on alkaline phosphatases. Int J Microbiol and Bioinformatics 2012; 2(Issue 1): 1-4.
16. Meisel F, Schwan C, Luedeman J, John U, Kroener HK, Kocher T. Magnesium deficiency is associated with periodontal disease. J Dnt Res 2005; 84(10): 937-41.
17. Grober U, Joachim S, Klaus K. Magnesium in prevention and therapy. *Nutrients* 2015; 7(9): 8199-226.
18. Volpe SL. Magnesium in disease prevention and overall health. Adv Nutr 2013; 4: 378S-383S.
19. Wilhelm JD, Markus K. Magnesium basics. Clin Kidney J 2012; 5(Suppl 1): i3-i14.
20. Farah HS, Ali AA, Gaber MS. Explanation of the decrease in alkaline phosphatase (ALP) activity in hemolysed blood samples from the clinical point of view: In vitro study. J of Biological Sci 2012; 5(2): 125-8.

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 56/DIKTI/Kep./2012.

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