

Original article

Zn as the Factor of Alkaline Phosphatase in Periodontal Patients consuming *Minangkabau* food in West Sumatra, Indonesia

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Abstract

Background: Periodontal disease is preceded by an inflammation in the gingiva surrounding the teeth called gingivitis. It is an untreated gingivitis causing hard tissue damage (alveolarbone). This inflammation increases the activity of ALP enzyme. Zinc is an Alkaline Phosphatase co-factor. **Aim:** This study aims at proving the connection between Alkaline Phosphatase (ALP) and zinc within the food of the *Minangkabau* people in West Sumatra, Indonesia. 60 samples are involved in this study. ALP level is tested by using ELISA technique. Zinc level in *Minangkabau* food is measured by Food Frequency Questionnaire (FFQ). Data is analyzed by means of univariate to describe each variable, Kolmogorov Smirnov Test is used to see the normal distribution ($p > 0.05$). **Result:** Pearson correlation is conducted to see the correlation between ALP level and zinc level in *Minangkabau* food. The study reveals that ALP activity is higher in mild periodontitis compared to mild gingivitis and healthy control. Zinc level is significantly lower in mild periodontitis than in mild gingivitis and healthy control individuals. The relation between ALP and zinc level indicates strong correlation with positive direction ($r = -0.884$). **Conclusion:** Zn decreases ALP activity and shows that zinc complement has both preventive and curing effects in periodontitis patients.

Keywords: Alkaline Phosphatase; Periodontal Patients; Zinc; and *Minangkabau* food.

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Introduction

Micronutrients such as zinc play essential roles in regeneration for coping with oxidative stress and for adequate immune response. Therefore, this element is essential to maintain health. Micronutrients can cause diseases through toxicity, deficiency, and imbalance. Studies have shown that the decrease of zinc level could be a contributing factor in many inflammatory conditions¹. Consequently, deficiency in zinc level may impair regenerative capacity. Zinc deficiency also impairs both innate and acquired immunity (e.g., downregulation of phagocytosis by macrophages and neutrophils, natural killer cell activity, generation of oxidative burst, antibody responses, and the numbers of cytotoxic T cells.² Zinc and magnesium are Alkaline Phosphatase (ALP) co-factors. The

measurement of ALP enzyme in periodontal disease may indicate the severity of inflammation. Zinc is an essential nutrient required in the body of humans and animals for many physiological functions, including immunity, antioxidant, growth, and reproduction. Zn deficiency affects the immune defense of the body and muscles. The gingival tissue which supports the teeth is a muscle in the oral cavity. The oral gingival tissue consists of gingival fibers that are circular, dentogingival, dentoperiosteal, alveologingival, and alveolar groups which are beneficial to tighten firmly the gingiva ledge of the tooth so as to provide the necessary rigidity to resist the forces of mastication without the need to avoid tooth surfaces and to unite the free marginal gingiva and the root cementum of the adjacent attached gingiva.³

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Periodontal disease begins with gingivitis in soft tissue, hard tissue damage subsequent the alveolar bone called periodontitis. The activity of periodontal disease is caused by an increased inflammation and a bone turnover rate.⁴ Bone turnover is a process in which the circulation of bone osteoclasts breaks down bone, and osteoblasts work rebuilding bones. In periodontitis, boneturnoverincreases the damage through osteoclasts in bone.⁵In such condition, theproduction of ALP is high.²Alkaline Phosphatase(ALP) is a homodimericmet alloenzyme that catalyzes the hydrolysis of phosphate monoesters to become nonspecific inorganic phosphate and alcohol. The structure dimension of $100\text{\AA}\times 50\text{\AA}\times 50\text{\AA}$ to the actives ites within 30A of each opposite side of the molecule. Each active site has 2bonds Zn ions(Zn1 and Zn2) and a Mgions.⁶ALP concentration is able to indicate the location of the inflammation. The next stage is when the bone forming osteoblasts, and periodontal ligament fibroblasts regenerate, ALP production increases.² Zinc, as an ALP co-factor will affect ALP activity. Zinc defficiency would negatively affect bone - related enzyme , ALP , and other bone - related minerals. It is proved by the study of Cho et al (2007)⁷on 30 Sprague Dawley rats which were assigned to one of the three different Zndietary groups, such as Znadequate (ZA, 35 mg/kg), pair fed (PF, 35 mg/kg), Zndeficient (ZD, 1 mg/kg) diet, and fedfor 10 weeks. The result shows that ALP activity in the plasma of the ZD rats was significantly decreased compared to the ZA rats. Another study by Orbaketal (2007)⁸found that periodontal status was better in group rats fed with a zinc-containing diet than in group zinc-deficient rats. Thus, it can be suggested that inadequate intake of zinc may lead to suppressed immunity along with increased oxidative stress and poor regenerative capacity in an individual, which can predispose to periodontitis.

Method

This study is a cross sectional comparative study, dependent and independent variable were examined concomitantly to see the magnesium level in 3 groups of sample based on Periodontal Disease Index (PDI) by Ramfjord. Samples were taken by consecutive sampling technique based on exclusion and inclusion criteria. The sample consisted of the *Minangkabau* people who consume *Minangkabau* food daily. The excluding criteria was: the consumption of antibiotics and anti-inflammatory during the last 3 months, smokers, pregnant, and women in menstruation have systemic disorders

such as diabetes melitus, and have had previous periodontal treatment during the last 3 months . The first examination of periodontal tissues used PDI and a periodontal probe instrument. This tool was used to measure the depth of pockets. Normal depth of the pockets around 0-3 mm . Scores Periodontal Disease Index 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 of 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 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. The selection of 3 groups of PDI is to examine changes on healthy, mild gingivitis, mild periodontitis groups. The clinical characteristics of mild gingivitis are erythema and minimal bleeding on probing, the same as the characteristics of stage II of gingivitis, the early lesion. The comparison of ALP level on the three groups illustrating the increase of ALP level is detectable in 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 were 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. 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 in sterile test tubes from each person between 8.00 and 12.00 am. All subjects were requested to avoid eating and drinking 1 hour before sampling. GCF was collected with Absorbing Paper Strip method. Patients sat on dental chair and rinsed mouths with a solution of 2% klorheksidin. Paper points was inserted by using the superficial intracrevicular technique and was left for

3 minutes then each tube was frozen at -20°C until it was sent to biochemistry laboratory. Another GCF collection was done only in patient group during 30 days. The collection of GCF was followed by the filling in of FFQ questionnaire. Each participant answered a food frequency questionnaire regarding *Minangkabau* food. Zinc level measurement was carried out from FFQ in 220 kinds of *Minangkabau* food by way of cooking like curry, frying, boiling, grilling, and stir-frying. Then 60 saliva samples were sent to biochemistry lab at one time to avoid the problems of several setting up of the unit. The samples were analyzed using Elisa Kit for ALP, *homo sapiens* (Human), sE91472Hu with detection range 3.12-200 ng/ml and sensitivity 1.36 ng/ml USCN product by spectrophotometer variant hemoglobin testing analyzer called Bio rad. The measurement of ALP in GCF collected from gingival pocket from three groups was tested using ELISA sandwich method. After the termination of the ELISA reaction, ELISA reader immediately carried out with maximum wavelength of 450 nm for 30 minutes. The number coming out of the ELISA Reader is absorbent figures which should be compared with the help of standard chart was made after examination by ELISA Reader, the turn into numbers of ALP. Laboratory test performed at the biomedical laboratory of the Medical Faculty of Andalas University, Padang Indonesia with the best quality guaranteed and personnel who are competent in their respective field. The tools had to be calibrated first by following standard laboratory procedures on a periodic basis by the Quality Control System to determine the price of coefficient of variation (CV). Finally results were analyzed in each 3 groups by SPSS statistically software using Pearson correlation test.

Ethical Clearance: All subjects were informed of the purpose and informed consents were obtained from all the individuals and local ethical committee approval that was in accordance with the latest update of the Helsinki declaration.

Results

Study subjects consisted of 14 men (average age=21,15 years, std. deviation=31,18) and 46 women (average age=23,39 years, std. deviation=4,38). 60 GCF samples were obtained which consist of 20 healthy persons, 20 mild gingivitis patients, and 20 mild periodontitis patients. According to the following Table 1 there is a significant difference between ALP level in mild periodontitis and mild gingivitis as well as healthy group based on Periodontal Disease Index ($p < 0.05$).

Table 1: the difference of Alkaline Phosphatase level (ng / dl) in Gingival crevicular Fluid with Periodontal Disease Based on PDI

| Enzyme | PDI | f | Mean | SD | p |
|--------|--------------------|----|--------|-------|------|
| ALP | Healthy | 20 | 35,78 | 29,69 | 0,00 |
| | Mild Gingivitis | 20 | 132,92 | 29,33 | |
| | Mild Periodontitis | 20 | 235,74 | 25,08 | |
| Total | | 60 | 134,81 | 86,85 | |

According to Table 2, there is a significant difference between GCF zinc levels in mild periodontitis and mild gingivitis as well as healthy group ($P < 0.05$).

Table 2: the difference of Zinc level (mg) from FFQ in 220 kinds of *Minangkabau* 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 |

Based on table 2. there are significant differences in the levels of Zinc level on the terms of the PDI group, which is highest in healthy control with average = $7,09 \pm 1,12$ mg . The table above shows that mild gingivitis patients are likely to have reduced levels with average = $5,25 \pm 0,50$ mg. In mild periodontitis with average = $3,78 \pm 0,54$ mg. The next table shows the correlation of ALP and Zinc and the correlation of MMP-8 and Zinc

Table 3. the correlation Analysis of ALP and zinc level in *Minangkabau* food based on FFQ

| Level of ALP | Level of Zinc | R | P |
|--------------|---------------|--------|-------|
| | | -0,884 | 0,000 |

Statistic analysis using Pearson correlation test shows that coefficient of Pearson correlation (r) is $-0,884$ with significance level (p) 0,000 ($p < 0,05$) between ALP enzyme and Zinc.

Discussion

The results of the study show that the zinc level in Gingival Crevicular Fluid (GCF) of individuals with periodontitis is lower than that of gingivitis group and healthy. It is found that significant relation between the decrease of zinc in GCF increases the activity of ALP which is higher in mild periodontitis compared with mild gingivitis and healthy control. Zinc is involved in nucleic acid and protein

metabolism and in process of cell differentiation and replication. Zinc also protects against viruses by cell-mediated immunity stimulation. It increases the number of helper or effector T-cells, or precursors of antibody forming cells or increased suppressor cell activity.⁹ Additionally, zinc has antioxidant properties as it stabilizes the cell membrane structure and contributes to the structure of the superoxide dismutase and maintains the metallothionein in tissue concentrations. Zinc plays an important role in the functions of transcription factor, antioxidant defense system and DNA repair. Dietary deficiencies in the intake of zinc can contribute to single and double-strand DNA breaks and oxidative modifications to DNA that increase risk for cancer development.¹⁰ Zinc deficiency can have several effects but clinical assessment of mild zinc deficiency is difficult because many of the signs and symptoms are non-specific. Acrodermatitis enteropathica is a more severe childhood form of zinc deficiency, which manifests with periorificial (oral, anal, genital) and acral dermatitis, diarrhea, behavioral and mental changes, neurological disturbances and secondary bacterial and fungal infections. Zinc deficiency leads to altered metabolism of androgens, oestrogens and progesterone which impairs immunity indirectly.¹¹ This could be explained on the basis that zinc is an essential cofactor in enzymes like DNA polymerase and RNA polymerase and thus essential for DNA synthesis, protein synthesis, and cellular proliferation. Zinc deficiency has been shown to reduce osteoblastic activity, collagen and proteoglycan synthesis as well as alkaline phosphatase activity. Hence, deficiency in zinc could impair regenerative capacity.¹² Zinc's positive effects in established periodontal disease are also due to its action on calcium and calmodulin mediated processes such as mast cell degranulation, tissue damage induced by endotoxin and increased vascular permeability. These calcium-mediated events are responsible for much of the tissue destruction seen in periodontal disease. Regular (twice daily) use of a mouthwash that contains a 5% zinc solution inhibits plaque growth. However lower concentration or less frequent mouth washing are not particularly successful.¹³ A decrease in Zn status leads to a

decrease in the activity of some Zn dependent enzymes such as lactate dehydrogenase, glutamate dehydrogenase, ALP, pyridoxal phosphokinase and thymidine kinase. Thus Zn can also modulate protein, energy and nucleic acid metabolism by affecting enzymes containing or requiring Zn for their activities. Similarly, Zn deficiency can restrict cell proliferation. Earlier studies had shown that ALP activity decreases by as much as 48% in rats fed on a Zn deficient diet. In the present study we observed a significant decrease in activity of serum ALP in all the groups of malnourished children as compared to control. It is likely that low intake of Zn coupled with the high incidence of low protein intake are contributory factors for the reported low enzyme activity. Also a significant and positive correlation was obtained between serum Zn and ALP activity.¹⁴ According to the results, zinc level is significantly lower in mild periodontitis than in mild gingivitis and healthy control individuals. Prescribing zinc complement has both treatment and preventive effects in periodontitis patients.¹⁵

Conclusion

Based on the levels of zinc in *Minangkabau* food, significant correlation is found between the level of mild periodontitis zinc that is lower than mild gingivitis and healthy condition. Zinc is the co-factor of ALP, an enzyme that regulates turnover rate. Zinc also plays a role in the recovery of oxidative stress that occurs during periodontal tissue inflammation. Further research is suggested so as to measure the amount of zinc contained in food that is adjusted for the needs of daily zinc, and zinc level given in the form of food or supplements tablet to prove the role of zinc as an additional therapy in the treatment of periodontal disease.

Conflict of interest: None declared

Authors' Contribution:

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References:

1. Mujico JR, Pérez-de-Heredia F, Gómez-Martínez S, Marcos A. Malnutrition and Inflammation, Inflammation, Chronic Diseases and Cancer - Cell and Molecular Biology, Immunology and Clinical Bases. *InTech*; 2012.
2. Raggat, Liza Jane and Nicola C. Partridge . Cellular and Molecular Mechanism of Bone Remodelling. *J. Bio. Chem.* Published online May 25, 2010
3. Kumar, G. S. Orban's Oral Histology & Embryology. Elsevier Health Sciences. 2014
4. Al-Rawi, Nadia et al. Salivary Alkaline Phosphatase And Periodontal Disease. *MDJ*. Vol 8 No.2 2011
5. Seibel, Markus J . Biochemical Markers of Bone Turnover Part I: Biochemistry and Variability. *Clin Biochem Rev.* 2005 November; **26**(4): 97–122.
6. Satoshi Sekiguchi, Yasuhiko Hashida, Kiyoshi Yasukawa, and Kuniyo Inouye. tabilization of Bovine Intestine Alkaline Phosphatase by Sugars. *Biosci Biotechnol Biochem* **76** (1), 95–100, 2012
7. Prasad AS, Beck FW, Snell DC, Kucuk O. Zinc In Cancer Prevention. *Nutr Cancer* 2009;**61**:879-87. <https://doi.org/10.1080/01635580903285122> <https://doi.org/10.1080/01635580903285122> <https://doi.org/10.1080/01635580903285122>
8. Orbak R, Kara C, Ozbek E, Tezel A, Demir T. Effects of zinc deficiency on oral and periodontal diseases in rats. *J Periodontal Res.* 2007 Apr;**42**(2):138-43. <https://doi.org/10.1111/j.1600-0765.2006.00939.x> <https://doi.org/10.1111/j.1600-0765.2006.00939.x> <https://doi.org/10.1111/j.1600-0765.2006.00939.x>
9. Cho, Young-Eun, Ria-Ann R. Lomeda, Sang-Hoon Ryu, Ho-Yong Sohn, Hong-In Shin, John H. Beattie, and In-Sook Kwun. Zinc deficiency negatively affects alkaline phosphatase and the concentration of Ca, Mg and P in rats. *Nutr Res Pract.* 2007 Summer; **1**(2): 113–119 <https://doi.org/10.4162/nrp.2007.1.2.113> <https://doi.org/10.4162/nrp.2007.1.2.113> <https://doi.org/10.4162/nrp.2007.1.2.113>
10. Femiano F, Gombos F, Scully C. Recurrent Herpes Labialis: A Pilot Study of The Efficacy of Zinc Therapy. *Oral Pathol Med.* 2005;**34**:423–5. <https://doi.org/10.1111/j.1600-0714.2005.00327.x> <https://doi.org/10.1111/j.1600-0714.2005.00327.x> <https://doi.org/10.1111/j.1600-0714.2005.00327.x>
11. Dhawan, D. K, Vijayta D. Chadha. Zinc: A promising agent in dietary chemoprevention of cancer. *Indian J Med Res.* 2010 December; **132**(6): 676–682.
12. Ayodele JT, Bayero AS. Lead And Zinc Concentrations In Hair And Nail of Some Kano Inhabitants. *Afr J Environ Sci Technol.* 2009;**3**:164–70.
13. Joseph EP, Michael FM. Textbook of Natural Medicine . Elsevier Health Sciences. 2012
14. Jain A, Meena Varma, B.K. Agrawal, A. A. Jadhav. Estimation of serum zinc and alkaline phosphatase in malnourished children. *Curr Pediatr Res* 2008; **12** (1 & 2): 27-30
15. Nila Kasuma, The analysis of matrix metalloproteinase-8 in gingival crevicular fluid and periodontal diseases. *Indian Journal of Dental Research.* 2018 Volume : **29** Issue : 4: 450-454