Effect of Zinc Supplementation on Salivary MMP-8 Level in Male Wistar Rats with Experimental Periodontitis for a Better Dental Care

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Abstract

Periodontitis is the 6th most prevalent disease in the world with 11,2% prevalence and 743 million individuals infected. MMP-8 is one of the inflammation biomarkers which is the most MMP found in periodontal diseases. Zinc act as MMP-8 inhibitors, which makes it an alternative treatment to inhibits MMP-8. This research aims to analyze the difference between MMP-8 level in healthy rat saliva and experimental periodontitis rat saliva, and also to analyze the difference between MMP-8 level in periodontitis rat saliva with zinc supplementation and in periodontitis rat saliva without zinc supplementation. This research was done with 30 male Wistar rats as a sample divided into 3 different groups i.e. control group, periodontitis without supplementation group, and periodontitis with supplementation group. Every group saliva specimen was collected and MMP-8 level was examined using ELISA method. MMP-8 level was statistically tested using one-way ANOVA. Statistic test showed a significant difference between the group with $p<0,05$. This research concludes that zinc supplementation is effective in suppressing MMP-8 level in periodontitis.

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Introduction

Periodontitis is an inflammation process and progressive destruction of periodontal ligament caused by dental plaque pathogens such as *Treponema denticola, Tannerella forsythia,* and *Porphyromonas gingivalis*. This pathogen will induce the immune system to produce cytokines and increase the activity of osteoclasts. The advanced progress of gingivitis is also considered to cause this disease.[1,2,3,4,5]

Periodontitis is the 6th most prevalent disease in the world with 11,2% and about 743 million infected individuals. Periodontal disease is considered the main cause of tooth loss in the adult population across the globe, and as such, it affects the person's nutrition intake, quality of life and self-esteem. Periodontal disease also affects a country's finance as it increases the health service budget due to the high number of patients.[6,7] Tooth and oral problems prevalence in Indonesia accounts for 26% with periodontal disease ranking 2nd as the most prevalent disease in Indonesia.[8,9] The high incidence rate on a national and global scale caused by the first stage of periodontal disease progress is due to poor knowledge regarding this disease. Other predisposing factors are genetic, environment, individual (such as smoking, low salivary flow rate,) and social-economic.[6,10, 36,39,41]

Periodontitis decreases oral physiology efficiency and produces uncomfortable feelings while masticating food, tooth loss risk, and difficulty in pronouncing particular words. All of this affects a person's life quality. An individual suffering from periodontitis has a higher risk of contracting cardiovascular diseases such as atherosclerosis, myocardial infark, and stroke. Expectant women have a higher risk of developing periodontitis, which may suffer from preeclampsia, preterm birth, and low birth weight.[4,11,12,13,14, 38]

Patient history taking and clinical examinations such as gingival inflammation index, periodontal destruction index, calculus, and

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plaque index, probing depth, clinical attachment loss, bleeding on probing index, and tooth mobility index were performed to diagnose periodontal disease.[4,15] Using biological fluid like saliva can also act as one of the supported examinations, and may become the potential diagnosis medium for periodontal disease.[7,35,37,42,43]

Saliva contains local and systemic biomarker derivates. Because the sample collecting process is considered non-invasive, safe, and cheaper than other methods, makes it a potential diagnostic media for periodontal disease. Inflammation biomarkers such as IL-1β, IL-6, IL-8, MMP-8, TIMP-1, and TNF-α which correlates with oral disease and infections such as caries, gingivitis, and periodontitis can be found in saliva.[7,16,35,40,43.] Nowosielska et al found that chewing gum with added chitosan could also be used as a mechanical plaque control due to antibacterial effect, thus reducing caries risk and inhibiting the production of inflammation biomarkers such as MMP-8 and other pro-inflammatory cytokines.[39] MMP-8 are the most myriad MMP found in periodontitis. They are produced in established lesions, secreted by neutrophiles and are effective on degrading type 1 collagen, which is the most collagen contained in periodontal ligaments. MMP-8 level will increase within the severity of the disease, and decrease throughout the treatment process.[4,17,18,19,20]

Zinc (Zn) is the second most mineral found in the human body and plays an important role in keeping cell integrity, growth, and development. Zinc also acts as a cofactor for more than 200 enzymes and serves as an antioxidant for neutralizing bacterial toxins, anti-inflammation, and anti-apoptotic effects. In wound healing, zinc regulates auto the debridement process and the keratinocyte migration.[11,21,22] In cell regulation, zinc plays an important role in stimulating osteoblast bioactivity, bone formation, and forming collagen structure on periodontal tissue.[23,24] MMP-8 Overproduction causes numerous pathological conditions such as cancer, peripheral nerve injury, pathological bone resorption, skin, and respiratory tract inflammation, Crohn's disease, hypersensitivity reaction, Alzheimer, and periodontitis. Zinc acts as MMP-8's cofactor that affects enzyme reaction and as an inhibitor when there's an excess in this enzyme production. This condition makes zinc an alternative treatment for inhibits MMP-8 overproduction. [17,25]

Materials and methods

This study aims to analyze zinc supplementation's effect on MMP-8 level in rat saliva with periodontal disease. The design of the experimental study was a post-test only control group design. The total population was allocated using simple random sampling with a group categorized as the control group (C), experimental periodontitis with placebo treatment group (P), and experimental periodontitis with zinc treatment (Z), each group consisted of 8 rats. The population was adult male Wistar rats, 8 weeks old, weight 273,3 gram (± 21,4 gram). The study was conducted in Medical Faculty Andalas University, Padang, West Sumatera. The study was approved by the Committee of Research Ethics of the Faculty of Medicine Andalas University. Independent variables of this study are periodontitis and zinc supplementation. Dependent variables are MMP-8 level on rat saliva before and after zinc supplementation. Controlled variables of this study are population criteria, specimen collecting method, and experiment time

The samples were acclimatized for 1 week and were placed in a plastic cage according to the experimental group. Then, P and Z groups were inducted with periodontitis using wire ligatures on mandible incisive for two weeks. The ligatures had to be observed every day to retained their position subgingivally.[1,26,2,27] After 2 weeks of the induction period, ligatures were removed and zinc supplementation was administered orally 1time/day for 7 days.[28,29] Saliva was extracted using pilocarpine HCl 5mg/kg BW with intraperitoneal injection and was collected using a syringe for 40 minutes then was transferred to a microtube. The examination was conducted using an enzyme-linked immunosorbent assay kit (Elabsciences EELR0623).

Data from ELISA reader were analyzed using statistic software (SPSS version 17) the relationship between groups and salivary MMP-8 level was tested using a one-way ANOVA test.

Results

This experiment was a preliminary study that started from January to March 2018, the

samples were 24 Wistar rats with an average body weight of 264,3 gram (±20,4 gram). Saphiro Wilk normality test was done to determine data distribution, which indicated normal with p-value > 0,05. All the data were then analyzed using the one-way ANOVA test. Results from the one-way ANOVA test shows that the difference between groups was significant with p-value < 0,05. The highest salivary MMP-8 level was found in the P group with a value of 1246, 07 ±593,18 while the lowest was found in the C group with a value of 323,74 ±135,08. The relationship is described in the table below.

Group	MMP-8 saliva $\bar{x} \pm SD$	n	p
Control group (C)	323,74 ± 135,08	8	
Experimental periodontitis with placebo therapy group (P)	1246, 07 ± 593,18	8	.000
Experimental periodontitis with zinc supplementation group (Z)	407,30 ± 240,45	8	

Table 1. Salivary MMP-8 level between groups.

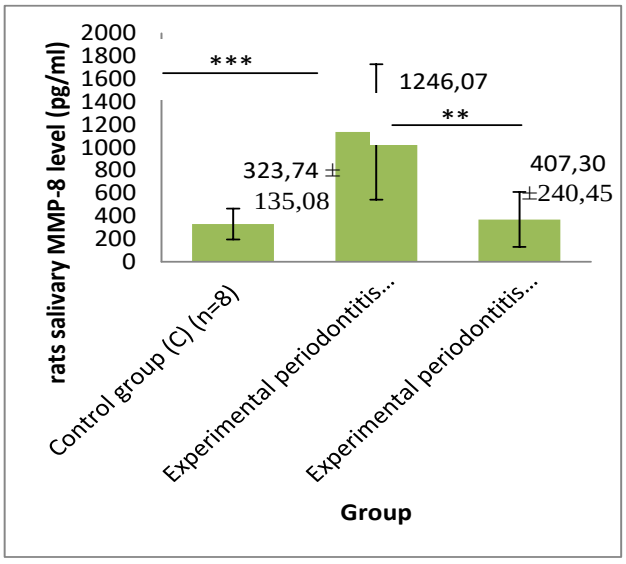

Figure 1. Post-Hoc Bonferroni test.

One-way ANOVA test followed with Post-hoc Bonferroni described there is a significant relationship between groups with p-value ***p<0,001 and **p<0,01. To see the significance between groups, the analysis continues with the Post-Hoc Bonferroni test described in the graphic.

Discussion

Periodontitis is one of the periodontal inflammations that can result in tissue degradation and the loss of teeth bone support. Periodontitis is a severe state that can lead to tooth loss.[34] Zinc is one of the nutritional components needed for growth and it also acts as an enzyme cofactor. This mineral can also stimulate some of the protein gene expressions needed for bone mineralization and collagen structure development.[30,31] On the inflammation process, T cell induces macrophage formation to eliminate foreign substances while the B cell maturation process is regulated by zinc.[31]

The difference between in rat salivary MMP-8 level is caused by collagenase activity produced by the periodontal pathogen. Stress can also enhance MMP-8 production.[19,32,33] Matrix metalloproteinase 8 is one of the Zn^{2+}*dependent endopeptidases* which can cause extracellular matrix degradation of periodontal tissues. On a normal condition, MMP-8 is produced for tissue regeneration and on a pathological condition, there is an overproduction as well as an enhanced activity of this enzyme[1,17,32], which is regulated by extracellular stimuli such as bacterial lipopolysaccharide, which can induce cytokine production interleukin-1β, TNF-α, and TGF-β. Enhancing neutrophile activity on tissue and stress factors experienced by the host could also contribute to the overproduction of MMP-8.[17,19,32,33,26]

Zinc supplementation given to rats after ligature induction will enhance the production of TGF-β1 as well as TIMP-1, which will inhibit MMP-8 production and reduce collagenase activity. On periodontitis followed with bone loss condition, zinc supplementation will suppress osteoclast differentiation and induce osteocalcin activity to enhance osteoblast mineralization.[1,30,32,31,26]

Conclusions

Based on the experimental results, it can be concluded that rats that were given zinc

supplementation during periodontitis could suppress salivary MMP-8 production. This suggests that zinc supplementation is effective in suppressing MMP-8 level in periodontitis.

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Declaration of Interest

The authors report no conflict of interest.

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