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Zinc Supplementation Effect on Salivary MMP-8 Level in Male Wistar Rats with Experimental Periodontitis

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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.***

Keywords:MMP-8 level, rat saliva, zinc supplementation, Wistar rats, periodontitis

I. 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.

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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 within 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 affectsa country's finance as it increases the health service budget duet o 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 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 and social-economic.[6],[10]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 sufferingfrom periodontitis has a higher risk ofcontracting cardiovascular diseases such as atherosclerosis, myocardial infark, and stroke. Expectant women with periodontitis can suffer from preeclampsia, preterm birth, and low birth weight.[4],[11],[12],[13],[14]

Patient history taking and clinical examinations such as gingival inflammation index, periodontal destruction index, calculus, and plaque index, probing depth, clinical attachment loss, bleeding on probing index, and tooth mobility index was 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] 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]. MMP-8 are the most myriad MMP found in periodontitis. They are produced in established lesions, secreted by neutrophile 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 toxin, anti-inflammation, and anti-apoptotic effect. 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 as an alternative treatment for inhibits MMP-8 overproduction.[17],[25]

II. METHOD

This study aims to analyze zinc supplementation effect on MMP-8 level on rat saliva with periodontal disease. The design of the experimental study was 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 its 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 one-way ANOVA test.

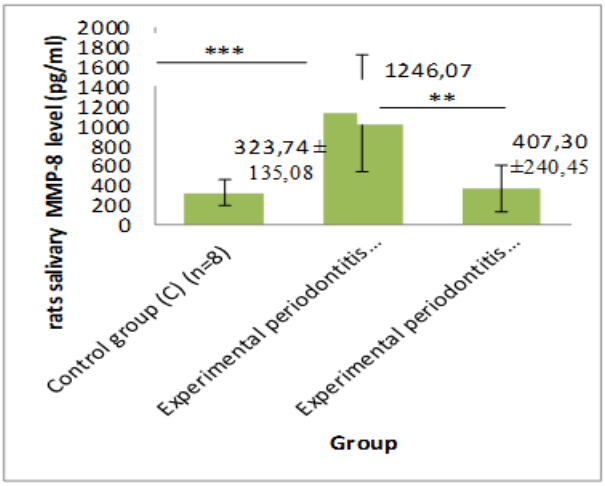
III. RESULT

This experiment was a preliminary study which 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 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 $\pm$593,18 while the lowest was found in the C group with a value of 323,74 $\pm$135,08. The relationship is described inthe table below

Table 1. Salivary MMP-8 level between groups

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

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 with the graphic below.



IV. DISCUSSION

Periodontitis is one of the periodontal inflammations which can result in tissue degradation and the loss of teeth bone support. Periodontitis is a severe state 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 periodontal pathogen. Stress can also enhance MMP-8 production.[19],[32],[33]Matrix metalloproteinase 8 is one of the Zn2+dependent endopeptidaseswhich 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 asan 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 enhancethe 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]

V. CONCLUSION

Based on these experimental results,it can be concluded that rats that were given zinc supplementation during periodontitis could suppress salivary MMP-8 production.

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REFERENCES

1. Balli, U., B.O. Cetinkaya, G.C. Keles, Z.P. Keles, S. Guler, M.U. Sogut, Z. Erisgin. 2016. Assessment of MMP-1, MMP-8, and TIMP-2 in Experimental Periodontitis Treated with Kaempferol. *Journal of Periodontal and Implant Science* 46(2):84-95. [CrossRef]
2. Molon, R.S.D., V.I. Mascarenhas, E.D. de Avila, L.S. Finoti, G.B. Toffoli., D.M.P. Spolidorio, R.M. Scarel-Caminaga, S. Tetradis, J.A. Cirelli. 2016. Long-Term Evaluation of Oral Gavage with Periodontopathogens or Ligature Induction of Experimental Periodontal Disease in Mice. *Clin Oral Invest* 20:1203-1216. [CrossRef]
3. Santos, B.F.E., E.Q.M. Souza, M.R.P.L. Brigagao, D.C. de Lima, L.A. Fernandes. 2016. Local Application of Statins in the Treatment of Experimental Periodontitis in Rats. *Journal of Applied Oral Science* 25(2):168-176. [CrossRef]
4. Newman, M.G., H.H. Takei, P.R. Klokkevold, F.A. Carranza. 2015. *Carranza's Clinical Periodontology*. 12th edition. Elsevier. Missouri.
5. Rivera, M.F., J.Y. Lee, M. Aneja, V. Goswami, L. Liu, I.M. Velsko, S.S. Chukkapalli, I. Bhattacharya, H. Chen, A.R. Lucas, L.N. Kesavalu. 2013. Polymicrobial Infection with Major Periodontal Pathogens Induced Periodontal Disease and Aortic Atherosclerosis in Hyperlipidemic ApoEnull Mice. *PLoS ONE* 8(2):e57178. [CrossRef]
6. Tonetti, M.S., S. Jepsen, L. Jin., J. Otomo-Corgel. 2017. Impact of the Global Burden of Periodontal Diseases on Health, Nutrition and Wellbeing of Mankind: A Call for Global Action. *Wiley Journal of Clinical Periodontology* DOI: 10.1111/jcpe.12732:1-7. [CrossRef]
7. Rathnayake, N., D.R. Gieselmann, A.M. Heikkinen, T. Tervahartiala, and T. Sorsa. 2017. Salivary Diagnostics Point-of-Care Diagnostics of MMP-8 in Dentistry and Medicine. *Diagnostics* doi: 10.3390/diagnostics7010007. 7(7):1-12. [CrossRef]
8. Infodatin, 2014. *Situasi Kesehatan Gigi dan Mulut.* September. Kemenkes RI Pusat Data dan Informasi. Jakarta.
9. Riskesdas, 2013. *Riset Kesehatan Dasar*. Badan Penelitian dan Pengembangan Kesehatan. Kemenkes RI. Hal 110 – 9.
10. Jordao, L.M.R., D.N. Vasconcelos, R. D. S., Moreira, M. D. C. M. Freire. 2012. Individual and Contextual Determinants of Periodontal Health in 12-Year-Old Schoolchildren in a Brazilian Capital City. *International Journal of Dentistry*:1-7. Article ID 325475, DOI:10.1155/2012/325475. [CrossRef]
11. Najeeb, S., M.S. Zafar. Z. Khurshid, S. Zohaib, and K. Almas. 2016. The Role of Nutrition in Periodontal Health: An Update. *Nutrients* DOI: 10.3390/nu8090530. 8(530):1-18 [CrossRef]
12. Manea, Al., C. Ciobanu, M. Nechifor. 2013. Influence of Smoking on the Salivary and Blook Concentrations of some Bivalent Cations in Patients with Chronic Periodontitis. *International Journal of Medical Dentistry* 3(1):57-64
13. Aly, L.A., H. El-Menoufy, R.T. Elsharkawy, M.Z. Zaghloul, D. Sabry. 2015. Maternal Chronic Oral Infection with Periodontitis and Pericoronitis as a Possible Risk Factor for Preeclampsia in Egyptian Pregnant Women (Microbiological and Serological Study). *Future Dental Journal* 1:23-32. [CrossRef]
14. Simona, G., M. Silvia, B. Carina. 2014. Quality of Life Regarding Patients with Periodontal Disease in Iasi, Romania. *Procedia-Social and Behavioral Sciences* 127:15-20. [CrossRef]
15. Reddy, S. 2011. *Essentials of Clinical Periodontology and Periodontics.* 3th edition. Jaypee Brothers Medical Publishers(P) Ltd. New Delhi. [CrossRef]
16. Prodan, A., H. Brand, S. Imangaliyev, E. Tsivtsivadze, F. van der Weijden, A. de Jong, A. Paauw, W. Crielaard, B. Keijser, E. Veerman. 2016. A Study of the variation in the Salivary Peptide profiles of Young Healthy Adults Acquired Using MALDI-TOF MS. *PLoS ONE* 11(6):1-15. [CrossRef]
17. Kasuma, N., F. Oenzil, and N.I. Lipoeto. 2016. Correlation Between Matrix Metalloproteinase 8 in Gingival Crevicular Fluid and Zinc Consumption. *Pakistan Journal of Nutrition* 15(1):72-75. [CrossRef]
18. Akbari, G., M.L.V. Prabhuji, B.V. Karthikeyan, K. Raghunatha, R. Narayanan. 2015. Analysis of Matrix Metalloproteinase-8 Levels in Gingival Crevicular Fluid and Whole Mouth Fluid Among Smokers and Nonsmokers Using Enzyme-linked Immune-sorbent Assay and a novel Chair-side Test. *Journal of Indian Society of Periodontology* 19(5):525-530. [CrossRef]
19. Ridwan, R.D. 2015. The Influence of Adhesin Protein from *Aggregatibacter actinomycetemcomitans* on IL-8 and MMP-8Titer in Aggressive Periodontitis. *Majalah Kedokteran Gigi* 48(1):39-42. [CrossRef]
20. Cekici, A., A. Kantarci, H. Hasturk, and T.E. van Dyke. 2014. Inflammatory and Immune Pathways in the Pathogenesis of Periodontal Disease. *Periodontol 2000* 64(1):57-80. [CrossRef]
21. Sadegh, A.A.M., G. Rezvaneh, E.M. Shahroo, A. Mojgan, K. Azam, R. Shahram, S.A. Reza, M. Nafiseh. 2016. Zinc Supplementation Effect on Orthodontical Tooth Movement in Rats. *Dental Press Journal of Orthodontics* 21(2):45-50. [CrossRef]
22. Zhang, C., X. Lu, Y. Tan, B. Li, X. Miao, L. Jin, X. Shi, X. Zhang, L. Miao, X. Li, L. Cai. 2012. Diabetes-Induced Hepatic Pathogenic Damage, Inflammation, Oxidative Stress, and Insulin Resistance was Exacerbated in Zinc Deficient Mouse Model. *PloS ONE* 7(12):1-7. [CrossRef]

23. Chou, J., J. Hao, H. Hatoyama, B. Ben-Nissan, B. Milthorpe, M. Otsuka. 2013. The Therapeutic Effect on Bone Mineral Formation from Biomimetic Zinc Containing Tricalcium Phosphate (ZnTCP) in Zinc-Deficient Osteoporotic Mice. *PLoS ONE* DOI: 10.1371/journal.pone.0071821 8(8):1-8. [CrossRef]
24. Praptiwi, E. Sulistyowati, Kustiyono. 2009. Pola Makan dan Pertumbuhan Bobot Tubuh Tikus yang Diinokulasi *Porphyromonas gingivalis* Sebelum dan Sesudah Terjadinya Periodontitis. *Media Medika Indonesiana* 43(5):229-233.
25. Singh, T., O.A. Adekoya, and B. Jayaram. 2015. Understanding the Binding of Inhibitors of Matrix Metalloproteinase by Molecular Docking, Quantum Mechanical Calculations, Molecular Dynamics Simulations, and a MMGBSA/MMBappl Study. *The Royal Society of Chemistry* 11:1041-1051. [CrossRef]
26. Danielsen, P.L., A.V. Holst, H.R. Maltesen, M.R. Bassi, P.J. Holst, K.M. Heinemeier, J. Olsen, C.C. Danielsen, S.S. Poulsen, L.N. Jorgensen, M.S. Agren. 2011. Matrix Metalloproteinase-8 Overexpression Prevents Proper Tissue Repair. *Surgery* 150(5):897-906. [CrossRef]
27. Theodoro, L.H., J.R. Pires, L.A. Fernandes, E.C.G. Junior, M. Longo, J.M. de Almeida, V.G. Garcia. 2015. Effect of Antimicrobial Photodynamic Therapy on Periodontally Infected Tooth Sockets in Rats. *Lasers Med Sci* 30:677-683. [CrossRef]
28. Hanafi, P., S.F. Muis, S. Hadisaputro, Suryono. 2015. Suplementasi All-Trans Asam Retinoat (ATRA) dan Zink Sulfat pada Periodontitis. *Jurnal Kesehatan Masyarakat* 11(1):74-79. [CrossRef]
29. Ozsoy, N., A. Can, O. Mutlu, N. Akev, R. Yanardag. 2012. Oral Zinc Supplementation Protects Rat Kidney Tissue from Oxidative Stress in Diabetic Rats. *Kafkas Univ Vet Fak Derg* Article Code: KVFD-2011-5650 18(4):545-550 [CrossRef]
30. Bortolin, R.H., B.J.D.G.A. Abreu, M.A.G. Ururahy, K.S.C. de Souza, J.F. Bezerra, M.B. Loureiro, F.S. da Silva, D.E.D.S. Marques, A.A.D.S. Batista, G. Oliviera, A.D. Luchessi, V.M.G.D.M. Lima, C.E.S. Miranda, M.V.L. Fook, M.D.G. Almeida, L.A. de Rezende, A.A. de Rezende. 2015. Protection Against T1DM-Induced Bone Loss by Zinc Supplementation: Biomechanical, Histomorphometric, and Molecular Analyses in STZ-Induced Diabetic Rats. *PLoS ONE* DOI:10.1371/journal.pone.0125349 10(5):1-18. [CrossRef]
31. Solomons, N.W., 2013. Update on Zinc Biology. *Annals of Nutrition and Metabolism* 62(1):8-17. [CrossRef]
32. Desarda, H., S. Gaikwad. 2013. Matrix Metalloproteinase & Implication in Periodontitis- A Short Review. *Journal of Dental & Allied Sciences* 2(2):66-70. [CrossRef]
33. Ridwan, R.D., 2012. The Role of *Actinobacillus actinomycetemcomitans* Fimbrial Adhesin on MMP-8 Activity in Aggressive Periodontitis Pathogenesis. *Majalah Kedokteran Gigi* 45(4):181-186. [CrossRef]
34. Kasuma, N., F. Oenzil, E. Darwin, Y Sofyan. 2018. The Analysis of Matrix Metalloproteinase-8 in Gingival Crevicular Fluid and Periodontal Diseases. *Indian Journal of Dental Research* 29(4):450-454. [CrossRef]