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To cite this article: W D Damaiyanti *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **217** 012057

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Expression Of Runx2 And Osteoblast Cell On The Periodontal Of Diabetes Mellitus Wistar Rat With Diet Extract Lemuru Fish Oils Treatment

W D Damaiyanti^{1*}, K Parisihni¹, D Mulawarmanti¹, H Kurniawan², Widyastuti²

¹Oral Biology Department, Faculty of Dentistry, Universitas Hang Tuah, Jl Arif Rahman Hakim 150, Surabaya Indonesia

²Periodontic Department, Faculty of Dentistry, Universitas Hang Tuah Jl Arif Rahman Hakim 150, Surabaya Indonesia

* damaiyanti@hangtuah.ac.id

Abstract. The purpose of this study was to find an innovative strategy for controlling progresivity of bone destruction on periodontal that caused by diabetic condition. Methods: Wistar rats sample are divided into 4 group; negative control group, 3 group with lemuru fish oil supplementation (4ml/kg of weight t, 8ml/kg of weight and 16ml/kg of weight). One week before treatment all group induced with STZ 65ml/KgBB and nicotinamide 110ml/KgBB to produced diabetes conditions. Immunohistochemistry slide of periodontal tissue was prepare after 3 weeks therapy. RUNX2 was count using HSCORE index, and osteoblast cell amount was count using image raster by optilab program. Results: Statistical analyses demonstrated a significant increase of RUNX2 expression in negative control group compare to treatment group ($p < 0,05$), and in treatment group showing less of RUNX2 expression ($p < 0,05$). Meanwhile Osteoblast cell amount was found increased and has significant difference in group 8ml/kg among other groups ($p < 0,05$) and lower osteoblast cell result in control group. The result lead to another mechanism that may involved in bone formation to induced osteoblast cell proliferation beside trancript factor RUNX2 which showed by treatment group of 8ml/kgWeight. Conclusion: The stimulation of osteoblast cell in diabetic condition can be induced by lemuru fish oil treatment, which regulated by another pathway mechanism beside trancript factor RUNX2.

Keywords : Bone destruction by diaabetic condition, RUNX2, Osteoblast, Lemuru Fish oil

1. Introduction

Diabetes mellitus (DM) is one of the major health problems, data from global studies shows that the number of people with diabetes mellitus in 2011 has reached 366 million people. If no action is taken, this number is expected to increase to 552 million by 2030 [1]. Periodontitis is the sixth most common complication caused by diabetes mellitus. Clinical studies have shown a higher prevalence of periodontitis in patients with diabetes mellitus [2]. Type 2 diabetes mellitus (T2DM) is a disorder that involved both metabolic and vascular components. It is characterized by hyperglycemia due to defective insulin function, many factors contribute to the onset and development of diabetes complications. Studies indicate that control of inflammatory processes may be related to novel approaches in treating this disorder [3].

The cellular and molular mechanism responsible for the cycle of diabetes and periodontitis is a condition of uncontrolled hyperglycemia, resulting in accumulation of glycation proteins called Advanced glycation end products (AGEs) in tissues including periodontal tissues [4]. AGEs and AGEs (RAGE) receptors bonds in monocytes cause an increase in the number of proinflammatory cytokines such as IL-1, TNF α , PGE2, IL-6 which can result in an excessive inflammatory response to periodontitis. [5].

The main goal of periodontal therapy is to stop the inflammatory process and destruction of the periodontal tissues. Some bioactive ingredients are added to obtain regenerative healing from



periodontists, proliferation of osteoblasts is one indication of bone healing [6]. Omega 3 Polyunsaturated fatty acids (PUFAs) have been known as anti-inflammatory agents [7].

Omega 3 has several metabolites such as resolvins and protectins, these two component have the ability to reduce pro-inflammatory enzyme production and reduce cytokine production including COX2, TNF α , and IL- β , which proofed that omega 3 can used as therapeutic agent for periodontal disease [7]. In the study of PUFA effects, it was reported that PUFA affects the regulation of serum TNF- α RUNX2, decreases in DHA administration, and there is an increase in PTH and IGF-1 in the administration of PUFA which causes an increase in RUNX2 translocation into the nucleus and stimulates osteoblast differentiation and ALP expression, and osteocalcin [8].

Lemuru fish (*Sardinella* sp) is a small pelagic fish that is often found in Indonesian oceans, there are two large populations of lemuru fish in Indonesia namely *Sardinella sirm* and *Sardinella longiceps* [9]. Fish oil byproduct of canning of lemuru fish taken from Muncar-Banyuwangi in June 1995 contained 26.79% omega 3 fatty acids with eicosapentaenoic acid (EPA) of 13.70% and docosahexaenoic acid (DHA) of 8.91%. EPA and DHA are a type of Omega 3 fatty acid that has been known to have the most potential influence on health [10]. The periapical bone resorption in the animals treated with fish oil for 14 days significantly reduced to the levels bone resorption [7]

Here, the purpose of this study was to find an innovative strategy for controlling progresivity of bone destruction on periodontal that caused by diabetic condition. In the experimental method, we compared the expression of RUNX2 and Osteoblast amount in periodontal with diabetic conditions with no treatment and with Lemuru fish oil treatment.

2. Experimental Method

This Study is laboratory experimental research used 40 male rats (*Rattus novvergicus* strain Wistar) aged around 3-4 months old and weighed about 200-250 gram as sampels with certains criteria. All experiments were approved by the Faculty of dentistry Animal Care Committee and performed in accordance with the guidelines of the Hang Tuah Council on Animal Care.

Induction of type 2 DM in rats was carried out by giving intraperitoneal nicotinamide around 240 mg / kg of weight dissolved in PBS liquid, 15 minutes later given a single dose of streptozotocin (STZ) of 65 mg / kg of weight. Previously rats were fasted overnight between 8-12 hours. Seven days after induction, rats experienced an increase in blood glucose levels of more than 126mg / ml and considered to have diabetes [11] [12].

Samples (Diabetic Rats) were divided into 4 groups, namely K0 (not given Lemuru fish oil extract), K1 (given Lemuru fish oil extract 4ml / kg of weight), K2 (given Lemuru 8 ml fish oil extract / Kg of weight), K3 (given Lemuru fish oil extract 16 ml / Kg of weight). Fish oil extract obtained from the lemuru fish oil waste treatment plant in Muncar, Banyuwangi district. Treatment rats and control rats were sacrificed on the 15th day after treatment with lemuru fish oil. Rats were sacrificed by placing them in glass tubes and using lethal dose of ether. Then the jaw decaputation was performed and inserted into the fixation solution and then the dead rats were buried.

Preparation of making histometry and immunohistochemistry preparations begins with mandibular decalcification using 10% EDTA solution for 73 days, then continued with tissue processing techniques with paraffin method, and performed Hematoxilin Eosin (HE) staining to calculate osteoblast cells. Whereas immunohistochemical preparations use monoclonal primary antibody, namely murine monoclonal antibody against RUNX2 molecule. Monoclonal antibodies are dissolved with TRIS-PBS 1: 200. For a 1 cm² network, 200 μ L of monoclonal antibodies are needed. Incubation is carried out 24 hours / overnight in a humid room [13].

Expressions RUNX2 calculated used HSCORE modification, with Olympus CX-21 microscope with optilab program, 400x magnification On preparation preparations seen in 3 fields of view, RUNX2 is calculated with HSCORE technique. The intensity of the score is assessed by categories: 0, absent; 1, weak; 2, moderate; and 3, intense. Then included in the formula HScore = $\sum (i + 1) \cdot P_i$, where i is the intensity score, P_i is the percentage of the number of cells colored, and 1 is the correction factor. The number of osteoblast cells in HE preparations is then calculated on the area along the alveolar bone, using a CX21 microscope, with 400x magnification, images were digitized with optilab camera

and analyzed with software by a calibrated examiner (BA) with no prior knowledge of the experimental design [14]

3. Results and Discussion

Figure 1 shows the expression RUNX2, HScore calculated using color intensity and proposition scores, which are then calculated using the formula. IHC staining of the image given is dark brown showing positive expression.

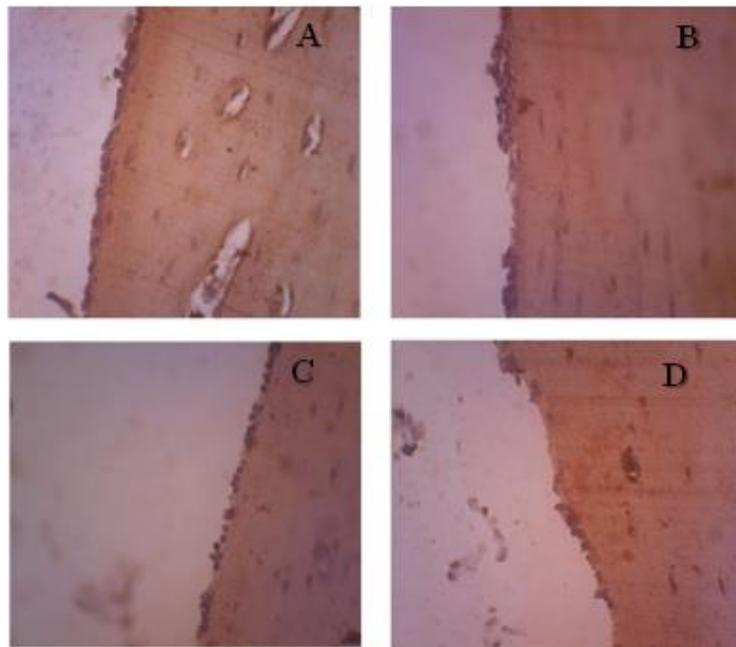


Fig 1. Immunohistochemistry of RUNX2, K0 (A), K1 (B), K2(C), K3 (D)

Hscore of RUNX2 show on table 1 obtained the highest RUNX2 score in the control group with an average of 24.3333 ± 9.05873 . The lowest average RUNX2 score was found in the K3 group (the Lemuru oil treatment group 16 ml / Kg of weight) of 4.3333 ± 1.43548

Tabel 1 HScore Values of RUNX2 Immunoreactive

Group	Mean \pm SD
KO	24.3333 ± 9.05873
K1	12.5000 ± 7.34228
K2	5.1667 ± 3.56328
K3	4.3333 ± 1.43548

Figure 2 shows of osteoblast cell among group. The number of osteoblasts counted using software, Osteoblast cells are cells on the edge of the alveolar bone periodontal tissue which are characterized by cuboidal cells, clustered along the alveolar bone.

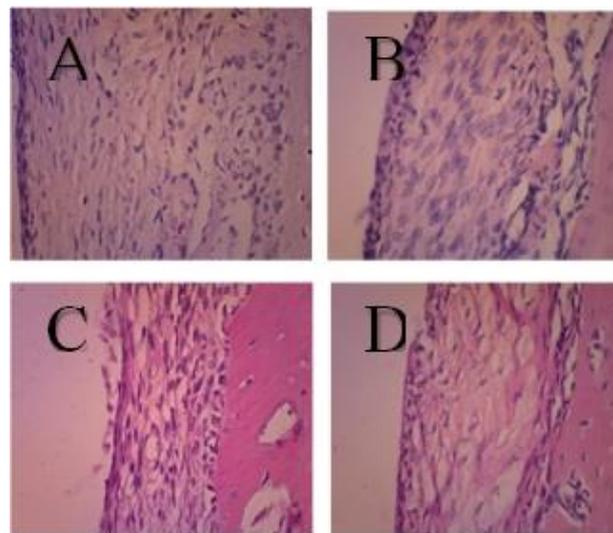


Fig 2. Hematoxylin Eosin (HE) of osteoblast cell along bone alveolar in periodontal

Based on table 2 below can be seen the largest number of osteoblasts in the treatment group 8 ml/kg with an average of 8.4 ± 2.066 . The lowest average number of osteoblasts was found in the negative control group at $3.4 \pm 1,647$.

Table 2. Number of Osteoblast cell among group

Group	Mean \pm Standar Deviation ($X \pm SD$)
K0	3.40 ± 1.647
K1	6.00 ± 1.333
K2	8.40 ± 2.066
K3	$6.40 \pm .843$

The data obtained were then analyzed to show differences between groups. Based on the above RUNX2 data above, the analyze show that there are significant differences between the control group and treatment group, indicated by the value of $p < 0.05$. Whereas in the Mann Whitney test between the 8ml / Kg of weight Lemuru fish oil group (K2) and the 16ml / Kg of weight Lemuru fish oil group (K3) for the RUNX2 score there was no significant difference where $p > 0.05$. The results of the analysis showed that the control group had the highest RUNX 2 score compared to other groups. Whereas in the lemuru fish oil group 16ml / Kg of weight the lowest RUNX2 score was obtained

Based on the data of osteoblasts above, it was analyzed and found that there were significant differences between the control group and the lemuru oil treatment group, indicated by the value of $p < 0.05$. Whereas in the Mann Whitney test between 4 ml / Kg of weight lemuru fish oil group (K1) and 16ml / Kg of weight lemuru fish oil group (K3), there were no significant differences where $p > 0.05$. Significant differences were found in the 8 ml / kg of weight lemuru fish oil treatment group (K2), with all groups. These results showed that in the treatment group 8 ml / Kg of weight had the highest number of osteoblasts and had significant differences compared to other groups

Transcript factor RUNX2 expression in control group was more active, while the osteoblast cell amount found lower. Meanwhile in treatment group was found lower of RUNX2 expression but showed higher osteoblast cell amount, this showed that treatment group was more active in bone

formation. RUNX2 is the main parameter that can indicate improvement in periodontal tissue bone damage. RUNX2 levels are very important because they can also show the progression of periodontitis. Increased RUNX2 shows that there is damage to the bone, resulting in repair by producing RUNX2 factor to trigger bone formation. Osteoblasts are mononucleated and specialized cells that are responsible for bone apposition. Osteoblasts and fibroblasts share a key functional similarity in that they both synthesize type I collagen matrix. Osteoblasts, however, distinguish from fibroblasts by expressing *Cbfa1* or *Runx2* that is a master switch for the differentiation of stem/progenitor cells into osteoblasts. Other osteogenesis genes include bone morphogenetic proteins, transforming growth factor- β [15]. There is another factor that can induce osteoblast. Osteoblast cell parameters can indicate improvement on bone destruction in periodontal tissue. Osteoblasts can indicate a new bone formation process, and also show healing in the bone healing process, because osteoblasts are cells that form a matrix that will later become mineralized into mature bone [16]. Which shown by Lemuru fish oil 8 ml / kg of weight lemuru fish oil treatment group (K2).

4. Conclusion

This study investigated the effect of lemuru fish oil diet on progressivity of periodontal destruction induced by diabetic condition. The study show there is another mechanism that may involved in bone formation to induced osteoblast cell proliferation beside trancript factor RUNX2. Lemuru fish oil diet proven to be able to stimulated osteoblast cell and can used as alternative treatment to reduce progressivity of periodontal destruction induced by diabetic

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Acknowledgement

We acknowledged LPPM Universitas Hangtuah, Internal research fund