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BMP2 expression on periodontal of wistar rats with stz - induced diabetes mellitus after giving diet extract fish oil

Hansen Kurniawan,[1] Dian Damaiyanti,[2]

1 *Department of Periodontics, Faculty of Dentistry, Universitas Hang Tuah Surabaya - Indonesia*
2 *Department of Oral Biology, Faculty of Dentistry, Universitas Hang Tuah Surabaya - Indonesia*

Abstract

Background : Diabetes mellitus (DM) is one of the most common metabolic disorders of high blood sugar levels. Lemuru fish is a resource of pelagic fish that has important economic value. Lemuru fish oil in addition to Omega-3 fatty acids that have been known as anti-inflammatory agent. Objective: To determine the effect of dietary extract of lemuru fish oil on BMP2 levels on periodontal tissue of Wistar rats induced with diabetes mellitus. **Materials and Methods:** The experiments were conducted with the Post Test Group design. Thirty male Wistar Rats were divided into four groups. Control group, Wistar induced STZ, but not treated. The first group of Wistar induced STZ and given lemuru fish oil extract 4ml / KgBB. The second group, Wistar induced STZ and given lemuru fish oil extract 8ml / KgBB and third group, Wistar induced STZ and given lemuru fish oil extract 16ml / KgBB. Wistar sacrificed. Then examined BMP2 with immunohistochemical methods. All data experiments were analyzed with Mann whitney ($p < 0.05$). **Results:** The results of this study showed that the control group compared to third group , it was found that in the control group and third group had significant differences. While in second group compared to third group, it was found that in second group and third group did not have significant differences. In first group compared with third group , it was found that in first groups and third group had significant differences. Similarly in second group compared with third group it was found that in second group and third group it was significant difference. From the statistical test it can be seen that in second group compared to third group there was no difference of effect. **Conclusion:** There was an effect of dietary extract of lemuru fish oil on BMP2 on periodontal tissue of Wistar rats induced by diabetes mellitus.

Keywords: BMP2, Wistar , STZ, Immunohistochemistry

INTRODUCTION

Diabetes mellitus (DM) is one of the most common metabolic disorders in the form of high blood sugar levels. Common symptoms are polyuria, polydipsia, polyphagia, and weight loss [1]. The prevalence of diabetes in the 45-54 year age group for urban areas in Indonesia ranks second at 14.7% [2]. The World Health Organization (WHO) estimates that diabetics in Indonesia in 2030 will reach 21.3 million. This will make Indonesia ranked 4th in terms of the number of diabetics after the United States, China, and India [2].

Indonesia, the prevalence of periodontal disease in all age groups reaches 96.58%. Nowadays, there are still many people who do not know that diabetes mellitus is closely related to periodontal disease, which is a chronic inflammatory disease in the tooth supporting tissue. Periodontitis has been identified as the sixth complication of diabetes. Some research states that diabetes is a risk factor for the prevalence and severity of gingivitis and periodontitis [3].

Periodontal disease affects humans almost all over the world and reaches 50% of the adult population. According to the results of a dental and oral health survey in East Java in 1995, periodontal disease occurred in 459 people out of 1000 population and more in rural than urban areas. In Indonesia periodontal disease ranks second main which is still a problem in society. The disease that attacks the gingiva and the supporting tissues of the teeth is a serious infectious disease and if not done proper care can result in tooth loss [4].

Lemuru is fish resource that has important economic value. Lemuru fish caught in Indonesian waters consist of several types , namely Sardinellalongiceps, S.aurita, S.leiogaster, S. sirm, and S.clupeoide. Among the five types of lemuru the most important is S.longiceps which is concentrated in the Bali sea,. Lemuru fish are also caught outside the waters of the Bali Sea, for example in the Madura sea and the Sunda sea[5].

***Corresponding author:**
Hansen Kurniawan
Department of Periodontics, Faculty of Dentistry, Universitas Hang Tuah Surabaya - Indonesia
Email:
hansen.kurniawan@hangtuah.ac.id

Lemuru fish oil obtained from the extraction of lemuru canning industry waste is one source of Omega -3. The results of this lemuru fish waste can also be processed into fish meal, which is the main source of protein in the preparation of livestock rations, especially chicken and pork, the use of lemuru fish oil in laying chicken rations can increase the added value of lemuru fish oil, on the other hand increase the economic value Omega-3 eggs. Lemuru fish oil in addition to containing Omega-3 fatty acids also contains Omega-6 fatty acids (Linoleic acid W6) which are needed for good egg quality that is to increase egg size [6].

BMP2 is one of the bmp proteins that plays an important role in bone and cartilage development [7]. It is involved in the hedgehog pathway, TGF beta signaling pathway, and in cytokine-cytokine receptor interaction [8].

Transforming growth factor beta (TGF-β) is a protein that is secreted to regulate the proliferation, differentiation and death of various cell types. All types of immune cells, including B cells, T cells and dendritic cells and macrophages, secrete TGF-β, which regulates proliferation, differentiation and activation by other cytokines. TGF-β is the main immunosuppressor associated with autoimmune, inflammation and cancer. TGF-β is a secretion protein consisting of three isoforms namely TGF-β1, TGF-β2 and TGF-β3. TGF-β1, is a major member of this signaling group whose role is widely known [9]. TGF β1 is what influences the secretion of BMP2 associated with osteoblast production and osteoclasts that affect alveolar bone resorption in patients with periodontitis [8].

MATERIAL AND METHODS

This research is classified as a true experimental laboratories research. The design of this study used the Post Test Only Control Group Design with experimental animals Rattus novergicus (wistar rat). Preparing wistar rats according to the sample criteria of 40 rats. Rats were adapted for 1 week in a 40 cm x 30 cm x 14 cm cage and placed in a room with enough air and light. Each cage contains 6 mice. Food is given by placing it in a small container and given every morning, afternoon, and night. While the drinks are given in 300 ml bottles which are equipped with small pipes and filled with boiled water. Experimental animals adapted for 1 week to get good general health and adaptation to the environment. Finally, weighing of experimental animals was carried out to meet the sample criteria [10.] DM induction in male white rats is done by giving nicotinamide around 240 mg per kg which is dissolved in PBS liquid 15 minutes and then given intravenous peritoneal streptozotocin injected with a single dose of 65 mg per kg in male white rats that have been fasted overnight between 8-12 hours, after 7 days the mice experienced an increase in blood glucose of more than 126mg / ml and were considered to have diabetes [11, 12].

In groups of K1, K2 and K3, Lemuru fish oil extract was administered at a dose of 4 ml / KgBB, 8ml / KgBB and 16 ml / KgBB for consecutive 14 days after rats had diabetes Treatment and control mice were sacrificed on the 15th day after treatment. The rat killing is placed in a glass tube and using ether in lethal doses. Then the jaw was decaputed and put into a fixation solution and the mice that had died were buried.

Preparations for histometry and immunohistochemistry test were begun by cutting the mucosa of the lower lip of the rat by including normal rat tissue. Then procced with the paraffin method technique [13].Excisional biopsy tissue was put into a formaldehyde buffer solution (10% formalin solution in the phosphate buffer saline at pH 7.0). When fixation is complete, the tissue is put into the aquadest solution for 1 hour to remove the fixation solution. After hat, put tissue cutting in to different alcohol concentration. The tissue becomes clearer and transparent, then put the tissue in to alcohol-xylol solution for 1 hour and then a pure xylol solution for 2x2 hours. After that the tissue is put in liquid paraffin for 2x2 hours [13].

The tissue is planted in solid paraffin which has a melting point of 56 to 58^0C, wait until the paraffin is solid. Tissue in paraffin is cut as thick as 5 microns in a microtome. Tissue cutting is affixed to a slide that has previously been smeared with polilisin as an adhesive. The tissue on the slide is heated in an incubator at temperature of 56 to 58 ^{0}C until the paraffin melts [13].

Immunohistochemical staining is done by deparafinizing first by washing tissue incisions successively: tissue incisions are washed with xylol 3 times each 5 minutes, washed with absolute ethanol for 5 minutes, washed ethanol 96% 2 times for 2 minutes, washed ethanol 90% 2 minutes, washed 80% ethanol 2 minutes. Then wash it under running water for 1 minute after it is dipped in aquadest. Then the tissue incision is inserted into a 3% H_2O_2 solution for 15 minutes, then washed with PBS (Phosphate buffer saline) pH 7.4 for 5 minutes and repeat again for 3 times. Then the slide is immersed in a citric acid solution and put in a microwave with a high temperature until a bubble occurs , temperature has been lowered to low temperature for 20 min. This process e to open the epitope of the material to be detected [13].

The next step is binding with primary antibodies, first wash the slide with PBS for 5 minutes and repeat again for 3 times. Then drops with backgroundsniper (Normal Swine Serum) to bind to the cell epitope that is not addressed. Wash with PBS for 5 minutes repeat again for 3 times. Then put the tissue incision into the primary monoclonal antibody, which is murine monoclonal antibody against BMP2. Monoclonal antibodies are dissolved with TRIS-PBS 1: 300. For 1 cm2 of tissue a 300 µL monoclonal antibody is required. Incubation is carried out for 1 hour per overnight in a humid room [13]. The next process the slides were washed with PBS pH 7.4 3 times each for 5 minutes. Incubation with secondary antibodies is anti-murine antibodies that have been biotinised (Dako kit) for 20 minutes incubation. Washed with PBS pH 7.4 for 5 minutes repeat again for 3 times. Then drop with HRP (strepavidin peroxidase conjugate) in the immunologic mark pen area to stand for 10 minutes. Washed with PBS pH 7.4 for 5 minutes repeat again for 3 times. Drop the DAB for 5 minutes, then rinse with water for 5 minutes. Then Enter into Mayer's Haematoksilin for 5 minutes, washed with running water for 5 minutes. Then washed with ethanol 80%, 90%, 96% respectively for 5 minutes. Followed by clearing using xylol for 5 minutes 3 times, mounting by closing it with a cover glass (deck glass) and glue with an entelan (poly lysine) [13].

Calculation of BMP2 expression using HSCORE calculation modification was carried out with Olympus CX-21 microscope with optilab program, 400x magnification. In preparations seen in 3 visual fields, BMP2 was calculated by HSCORE technique. The intensity of the score is assessed by category: 0, absent; 1, weak; 2, moderate; and 3, intense. Then it is entered in the formula HScore = Pi (i +1), where i is the intensity score, Pi is the percentage of the number of colored cells, and 1 is the correction factor [14].

The data obtained is then carried out statistical analysis. Analysis of BMP2 calculation data using the Mann Whitney test to determine differences in observations between dietary treatments of lemuru fish oil extract at doses of 4ml / kgBB, 8ml / kgBB, and 16ml / kgBB with the control group at 1 month observation [14].

RESULT

Based on this, the researcher wanted to know the effect of the administration of Lemuru fish oil extract on BMP2 expression in the periodontal tissue of Wistar rats induced by diabetes mellitus.

Table 1: Mann –whitney test Treatment group 2 (8 ml / KgBB) and treatment group 3 (16 ml / KgBB)

Group		N	Mean Rank	Sum of Ranks
Score BMP 2	Group 8 ml	10	7.10	71.00
	Group 16 ml	10	13.90	139.00
	Total	20		

Test Statistics[b]

	Score BMP2
Mann-Whitney U	16.000
Wilcoxon W	71.000
Z	-2.752
Asymp. Sig. (2-tailed)	.006
Exact Sig. [2*(1-tailed Sig.)]	.009[a]

a. Not corrected for ties.

b. Grouping Variable: kelompok

In the table above, the value of P = 0.006 <0.05 is obtained, so it can be concluded that the groups 2 and group 3 have significant differences.

Table 2: Mann–whitney test Treatment group 1 (4 ml / KgBB) and treatment group 3 (16 ml / KgBB)

Group		N	Mean Rank	Sum of Ranks
Score BMP 2	Group 4 ml	10	7.60	76.00
	Group 16 ml	10	13.40	134.00
	Total	20		

Test Statistics[b]

	Score BMP2
Mann-Whitney U	21.000
Wilcoxon W	76.000
Z	-2.384
Asymp. Sig. (2-tailed)	.017
Exact Sig. [2*(1-tailed Sig.)]	.029[a]

a. Not corrected for ties.

b. Grouping Variable: kelompok

In the table above, the value of P = 0.017 <0.05 can be concluded that groups 1 and group 3 have significant differences.

Table 3: Mann – Whitney test Treatment group 2 (4 ml / KgBB) and treatment group 3 (8 ml / KgBB)

Group		N	Mean Rank	Sum of Ranks
Score BMP 2	Group 4 ml	10	11.20	112.00
	Group 8 ml	10	9.80	98.00
	Total	20		

Test Statistics[b]

	Score BMP2
Mann-Whitney U	43.000
Wilcoxon W	98.000
Z	-.603
Asymp. Sig. (2-tailed)	.546
Exact Sig. [2*(1-tailed Sig.)]	.631[a]

a. Not corrected for ties.

b. Grouping Variable: kelompok

In the table above, the value of P = 0.546> 0.05 is obtained, so it can be concluded that the groups 2 and 3 have no significant difference.

Table 4: Mann –whitney test of the control group and the treatment group 3 (16 ml / kg)

Group		N	Mean Rank	Sum of Ranks
Score BMP 2	Control Group	10	5.70	57.00
	Group 16 ml	10	15.30	153.00
	Total	20		

Test Statistics[b]

	score BMP2
Mann-Whitney U	2.000
Wilcoxon W	57.000
Z	-3.728
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000[a]

a. Not corrected for ties.

b. Grouping Variable: kelompok

In the table above it is obtained that the value of P = 0.000 <0.05, it can be concluded that the control group and group 3 have significant differences.

Table 5: Mann –whitney Statistic test Control group and treatment group 2 (8 ml / KgBB)

Group		N	Mean Rank	Sum of Ranks
Score BMP 2	Control Group	10	6.80	68.00
	Group 8 ml	10	14.20	142.00
	Total	20		

Test Statistics[b]

	score BMP2
Mann-Whitney U	13.000
Wilcoxon W	68.000
Z	-2.938
Asymp. Sig. (2-tailed)	.003
Exact Sig. [2*(1-tailed Sig.)]	.004[a]

a. Not corrected for ties.

b. Grouping Variable: kelompok

In the table above, the value of P = 0.03 <0.05 can be concluded that the control group and group 2 have significant differences.

Table 6: Mann –whitney test statistic Control group and treatment group 1 (4 ml / KgBB)

Group		N	Mean Rank	Sum of Ranks
Score BMP 2	Control Group	10	6.85	68.50
	Group 4 ml	10	14.15	141.50
	Total	20		

Test Statistics[b]

	score BMP2
Mann-Whitney U	13.500
Wilcoxon W	68.500
Z	-2.901
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.004[a]

a. Not corrected for ties.

b. Grouping Variable: kelompok

In the table above, the value of P = 0.004 <0.05 is obtained, so it can be concluded that the control group and group 1 have significant differences.

DISCUSSION

BMP2 is one of the bmp proteins that play a role in bone and cartilage development. It is involved in the hedgehog pathway, TGF beta signaling pathway, and in cytokines-receptor interactions. BMP2 is also involved in the differentiation of heart cells and mesenchymal transition epithelium [7]. BMP2 and BMP7 are osteoinductive BMPs that induce osteoblast differentiation in various cell types [8]. In this study examination with Hematoxilen-Eosin (HE), then followed by immunohistochemical examination with BMP2 antibodies to see the experimental results from BMP2, which obtained P = 0.004 <0.05, it can be concluded that the control group and group 1 had significant differences . In the control group compared to group 3 obtained P = 0.000 <0.05 can be concluded that the control group and group 3 had significant differences. While in group 2 compared with group 3 were obtained P = 0.546> 0.05, it can be concluded that in group 2 and group 3 there was no significant difference.

Group 1 compared to group 3 the P = 0.017 <0.05, it can be concluded that in group 1 and group 3 had significant differences. Likewise in group 2 compared to group 3 P = 0.006 <0.05, it can be concluded that in group 2 and group 3 had significant differences. From the statistical test it can be seen that in group 2 (8 ml / KgBB) compared to group 3 (16 ml / KgBB) there was no difference in effect, but in the control group compared to groups 1, 2 and 3 had a significant effect. There is an effect of dietary administration of lemuru fish oil extract on BMP2 in the periodontal tissue of Wistar rats induced by diabetes mellitus.

CONCLUSSION

There is an effect of dietary of lemuru fish oil extract on BMP2 in the periodontal tissue of Wistar rats induced diabetes mellitus.

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Nil.

Conflict of Interest

The authors declare no conflict of interest.

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