ORIGINAL ARTICLE

Expressions of Osteoprotegerin and Receptor Activator of Nuclear Factor Kappa-B Ligand in Alveolar Bone Socket under Low Plasma Estrogen Level Condition

Agung Krismariono[1], Poernomo Agoes Wibisono[1], Noer Ulfah[1], Nina Agustina[1]*

[1]Department of Periodontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract:

Background: Decreasing of estrogen level can lead to alveolar bone loss. This bone loss reflects an imbalance between resorption and apposition. Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) is a key mediator in osteoclast activation, while Osteoprotegerin (OPG) is modulator of bone apposition. *Aim and Objectives:* To obtain the value of RANKL and OPG expressions in alveolar bone socket under condition of low plasma estrogen level. *Materials and Methods:* Sixteen female Wistar rats, aged 2-3 months divided into control and treatment group. Ovariectomy was done to treatment group. Seven days after ovariectomy, mandibular right incisor was extracted on each group. Fourteen days after extraction, immunohistochemical examination procedure was performed to determine expressions of RANKL and OPG level in alveolar bone socket. *Results:* The values of RANKL and OPG expressions in control group respectively were 5.13 ± 0.83 and 14.75 ± 1.39. In the treatment group, the values of RANKL and OPG expressions were 11.63 ± 1.6 and19.88 ± 0.40. Independent t-test showed that there was a significant difference between both groups ($p < 0.05$). *Conclusion*: Low plasma estrogen level condition can increase both RANKL and OPG expressions, but the increase was higher in RANKL expressions than in OPG expressions.

Keywords: Osteoprotegerin, Receptor Activator of Nuclear Factor Kappa-B Ligand, Bone Remodeling, Estrogen Deficiency

Introduction:

Everyone will experience aging process physiologically. In women, the aging process is faster due to reduction in the quantity of reproductive hormones, one of which is estrogen. Estrogen is a steroid hormone that plays a role in growth, differentiation, and function of a variety of cellular targets. One of its roles is in maintaining bone mass [1].

Bone undergoes continuous repair and remodeling processes. The remodeling process begins with resorption triggered by osteoclasts, followed with apposition conducted by osteoblasts [2]. Other than osteoclasts and osteoblasts, cytokines and growth factors play an important role in bone remodeling. In normal conditions, resorption and apposition occur in a balance to maintain bone mass [3]. Thus, bone mass loss may reflect that resorption activity is greater than apposition. One of the factors triggering bone mass loss is a decrease in the quantity of estrogen [4].

Estrogen has an important role in osteoblast differentiation. Estrogen can change osteoblast progenitors into mature osteoblasts, which then become osteocytes forming bone. This process cannot be separated from several essential proteins for bone remodeling, such as Osteoprotegerin (OPG), Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) and Macrophage-Colony Stimulating Factor (M-CSF) expressed by

osteoblasts, as well as Receptor Activator of Nuclear Factor Kappa-B (RANK) expressed by osteoclast progenitors [5]. Bone resorption begins a bond between RANKL and RANK, causing osteoclast differentiation. In addition, stimulation also occurs as a result of the bond between M-CSF expressed by osteoblasts and c-Fms receptor membrane, causing proliferation of osteoclasts. Both these processes lead to an increase in osteoclast activities [6]. Bone apposition, on the other hand, occurs through a bond between OPG and RANKL. The bond between the two proteins then makes RANK not bound on RANKL, thus inhibiting osteoclast activation [4].

Low plasma estrogen levels or estrogen deficiency, consequently, lead to impaired balance between resorption and apposition. Bone resorption activities triggered by osteoclasts become higher than osteoblast activities to form bone. In this condition, there is an increase in osteoclastogenesis that lead to an increase in bone resorption [7].

For these reasons, this research was aimed to determine the values of RANKL and OPG expressions in the alveolar bone under low plasma estrogen level condition. Based on the study of theory there is an assumption that RANKL and OPG expressions in the condition will be lower than in the normal condition. In addition, this research also aimed to reveal changes in OPG and RANKL expressions, so appropriate prevention or treatment can be determined.

Material and Methods:

This research was laboratory experimental study with post-test only control group design. This study received animal ethical clearance from the Bioethics Committee of Faculty of Dental Medicine of Universitas Airlangga, Surabaya (21/KKEPK.FKG/II/2016). The study was conducted at the Laboratory of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Brawijaya in February 2016. Animals were 16 female Wistar rats aged 2-3 months and weighed 200-250 grams. Those rats then were divided into two groups, a control group with 8 rats and a treatment group with 8 rats which underwent ovariectomy.

In the treatment group, ovariectomy was performed by opening peritoneal cavity under general anesthesia with an intra-muscular ketamine injection of 40 mg/kg (Kepro, Daventer, Holland) [8]. After the peritoneal cavity opened, transverse abdominal muscle, peritoneal space, and adipose tissue around the ovaries were opened. Ligase was performed on the distal uterine horns to take out the ovaries, both left and right. Then, it was stitched. Seven days after ovariectomy, the plasma estrogen level of these rats was observed for measuring the estrogen level under normal condition (< 20 ng/ml). Blood samples were taken from the tail vein of those rats, about 0.5 cc equivalent to 100 µl. Fourteen days after collecting blood samples, all of animals in both groups were sacrificed. Then extraction of right madibular incisors were done and alveolar bone of the right mandibular incisors were taken in a sagital direction.

The measurement of estrogen levels were conducted by using Enzyme-Linked Immuno-sorbent Assay (ELISA) test with a wavelength of 450 nm [9]. The histologic sample of mandibular alveolar bone were carried out for immuno-histochemistry tests on RANKL and OPG expressions. The data were statistically analyzed using independent t-test.

Results:

The measurement of estrogen levels using ELISA test were showed the following results:

Table 1: Mean ± SD of Estrogen Levels in Normal Rats and Ovariectomized Rats

Variable	Number	Normal	Ovariectomy
		Mean ± SD	Mean ± SD
Estrogen (ng/ml)	8	26.77±3.44	12.25±0.62

Immunohistochemical measurement showed the following results:

Table 2: Mean ± SD of RANKL and OPG Expressions in the Control Group and the Treatment Group having Ovariectomy

Variable	Number	Control	Ovariectomy
		Mean ± SD	Mean ± SD
RANKL	8	5.13±0.30	11.63±0.5
OPG	8	14.75±0.50	19.88±0.40

Furthermore, the results of independent t-test comparing the control group to the treatment group having ovariectomy, both on RANKL and OPG expressions demonstrated $p < 0.05$ indicating that significant difference.

Discussion:

Estrogen is a steroid hormone that has many functions for growth and differentiation, and other functions in various tissues. Estrogen is an important factor in maintaining the balance of bone metabolism, such as bone formation process by osteoblasts and bone resorption process by osteoclasts [1]. In postmenopausal women, estrogen levels in the blood plasma usually were below normal value, leading to an increasing bone loss. Consequently, to get rats with low plasma estrogen levels in this research, bilateral ovariectomy was performed by referring to a method used by Kumar [10]. With this technique, ovarian estrogen production could be stopped so that the

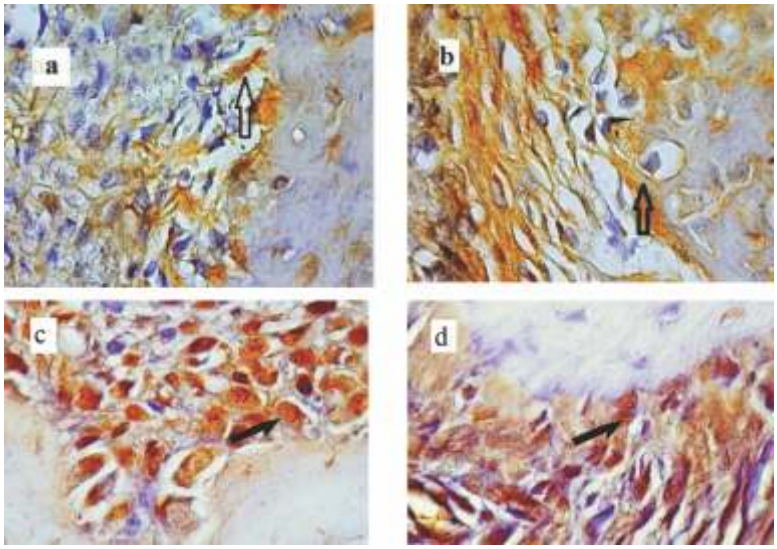

Fig. 1: Immunohistochemical RANKL Expressions in the Control Group (a) and in the Treatment Group Undergone Ovariectomy (b); and OPG Expressions in the Control Group (c) and in the Treatment Group Undergone Ovariectomy (d)

estrogen levels in plasma were below normal value.

Declining levels of estrogen occurred because of ovarian dysfunction as the main organ producing estrogen hormone. Estrogen hormone is produced not only by the ovaries, but also by cholesterol. Estrogen formation mechanism by cholesterol is through aromatase cycle involving aromatase enzyme of cytochrome P450. Aromatase will turn testosterone into estradiol to appear in the adrenal granulosa cells [11]. This is consistent with some researches reporting that there is an insignificant increase in estrogen levels after 6 months after ovariectomy. A research on ovariectomized rats conducted by Svetlana [12] showed a decrease in estrogen levels two weeks after ovariectomy and remained below normal levels by more than 9 weeks.

Thus, decreased estrogen levels will disrupt bone homeostasis process, which can lead to an increase in bone loss due to bone resorption greater than bone formation process. Bone resorption is triggered by an increase in both the number of osteoclasts (increasing osteoclast formation and decreasing osteoclast apoptosis) and the activities of osteoclasts [13].

Normal plasma estrogen levels physiologically play a role in osteoblast differentiation. The role of OPG as anti osteoclastogenesisis is more dominant. OPG binds to RANKL and inhibits RANKL to bind to receptor RANK. As a result, there will be an increase in the number and activity of osteoblasts [14]. Some researchers have also suggested that ovariectomy can result in a significant decrease in Bone Mineral Density (BMD) compared to control group. Similarly, Kalaiselvi also reported that a decline in BMD of women with osteoporosis compared to non-osteoporotic women [15].

Finally, the increasing values of RANKL and OPG expressions in the treatment group compared to the control group indicated a physiological compensatory mechanism as a result of the decreased estrogen levels. However, the increase in RANKL expressions was greater than the increase in OPG expressions. Therefore, the process of bone formation could be disturbed.

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[*]***Author for Correspondence:*** *Agung Krismariono, Department of Periodontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia Email: agung-k@fkg.unair.ac.id Tel/Fax: +62315020255/+6231.5020256*