Pathomechanism of Bone Mineral Density Decrease: A study of Role of Low Density Lipoprotein (LDL), Oxidized Low Density Lipoprotein (oxLDL) and Receptor For Activation of Nuclear Factor Kappa β Ligand (RANKL)

Sitti Rafiah[1*], Irene Edith Rieuwpassa[2], Uleng Bahrun[3]

Department of Anatomy, Faculty of Medicine Hasanuddin University, Indonesia
Department of Oral Biology, Faculty of Dentistry Hasanuddin University, Indonesia
Department of Clinical Pathology, Faculty of Medicine Hasanuddin University, Indonesia

[*]Corresponding Author : Sitti Rafiah, Department of Anatomy, Faculty of Medicine Hasanuddin University, Indonesia, email: sittirafiahdr@yahoo.co.id

ABSTRACT.

The research aimed to investigate the role of LDL, oxLDL and RANKL on the *Bone Mineral Density Decrease The research use the* cross sectional study of 78 women 30-60 years old who had the fasting blood glucose content, SGOT/SGPT, urea creatinine normal. Method used to assess the bone mineral density decrease was by using Dual Energy X-ray absorptiometry (DXA) which was measured on the lumbar spine and proximal femur, where as the measurement of LDL content by the photometry method using Petra 400 equipment, the measurement of oxLDL content and RANKL content using ELISA method. The data collected were processed by Mann-Whitney U test and chi-square test to perceive the difference on the normal bone mineral density group, osteopeni and osteoporosis. The research results indicated that the LDL content is not different on the three groups of bone mineral density ($p>0.05$), whereas the oxLDL content is not different between the normal group and osteopeni group ($p>0.05$), however, it is different between the normal group and osteoporosis group ($p<0,05$) and between osteopeni group and osteoporosis group ($p<0,05$). So is RANKL content is different between the groups ($p<0.05$) except between the osteopeni and osteoporosis ($p>0,05$). The data suggests a role of oxLDL and RANKL on decrease of bone mineral density. We concluded that oxLDL and RANKL contributes to decrease of bone mineral density, but otherwise LDL cholesterol to contribute does not significantly to decreased in bone mineral density.

Keywords: LDL, oxLDL, RANKL, bone mineral density.

INTRODUCTION

Along with increasing the quality of health services in developed and developing countries, the life expectancy increasing country's population.This means, will increase the population of elderly people. Diseases found in the elderly generally is degenerative chronic diseases. Osteoporosis become one of the disorders in the elderly that can reduce the quality of life.

An immunological study shows that there is a relationship between elevated cholesterol with the incidence of osteoporosis and indicate that immune responses play a role in the occurrence of bone mass reduction. Some researchers have osteoporosis patients who have high cholesterol levels and use of cholesterol-lowering drugs can reduce the risk of fractures. Research on animal shows, that activity can prevent oxLDL Receptor Activator of Nuclear Factor kappa B Ligand (RANKL) induced phosphorylation of ERK and JNK kinase. These results indicate that oxLDL, affect the differentiation of osteoclasts through inhibition of RANKL signaling pathways. This situation is probably related to the fact that atherosclerosis is accompanied by disturbances in bone remodeling and vascular causes of osteoporosis and vascular calcification.

MATERIALS AND METHODS

This study was an observational study with a cross-sectional design study in 78 women aged 30–60 years who examined LDL, serum calcium, Ureum, Creatinine, SGOT, SGPT, fasting blood glucose.

Inclusion Criteria

- Women 30 - 60 years old

- Never experienced a fracture and bone abnormality since birth.

- Does not suffer from joint disease (rematoid arthritis, SLE)

- Does not suffer: diabetes mellitus, chronic liver disease, chronic kidney failure, thyrotoxicosis.

- Don't smoke and drink alcohol

RESULTS

Research has been conducted on groups of women aged 30-60 years. After screening based on inclusion criteria, 78 research subjects were obtained.

The examination carried out is an examination of LDL cholesterol and serum calcium, examining bone mineral density in the lumbar spine and proximal femur by using the DXA method and subsequently examining Elisa oxLDL and RANKL.

Table 1. Distribution of samples based on age groups and bone mineral density

Age Group (years)	Bone Mineral Density (n=78)		
	Normal (n=27)	Osteopeni (n=43)	Osteoporosis (n=8)
30 – 44	11(44,0%)	14(56,0%)	0
45 – 51	13(48,2%)	14(51,8%)	0
52 – 60	3(11,5%)	15(51,7%)	8(30,8%)

The results showed that a decrease in bone mineral density was seen at 30 years of age and the incidence of osteoporosis increased with age (Table 1)

Table 2. Distribution of LDL, oxLDL, RANKL, serum calcium levels to Bone Mineral Density

Levels	Bone Mineral Density (n=78)								
	Normal (n=27)			Osteopeni (n=43)			Osteoporosis (n=8)		
	min	med	max	min	med	max	min	med	max
LDL (mg/dl)	61,0	113,0 ^{a1b1}	188,0	49,0	121,0 ^{a1c1}	196,0	57,0	148,0 ^{b1c1}	177,0
oxLDL (mU/L)	4,4	7,8 ^{a1b1}	13,0	4,0	8,8 ^{a1c1}	51,5	6,6	11,0 ^{b2c2}	14,1
RANKL (pmol/L)	3,0	11,0 ^{a1b1}	165,0	8,0	16,0 ^{a2c1}	180,0	8,0	23,5 ^{b2c1}	147,0
Ca serum (mg/dl)	8,0	9,0 ^{a1b1}	11,0	8,0	9,5 ^{a2c1}	11,0	13,0	14,0 ^{b2c2}	14,0

Description: min: minimum, med: median, max: maximal, Superscript a is a comparison of normal & osteopeni bone mineral density, Superscript b is a comparison of normal bone mineral density & osteoporosis, Superscript c is a comparison of osteopathic bone mineral density & osteoporosis.

The same superscript on the same line with the Mann-Withney U test showed no significant difference ($p > 0.05$), while the Superscript different from the Mann-Withney U test showed significantly different ($p < 0.05$)

Table 2 shows LDL levels did not differ in the three groups ($p > 0.05$), while oxLDL levels did not differ between the normal and osteopene groups ($p = 0.45$), but differed between the normal and osteoporosis groups ($p = 0.01$) and between the osteopenia and osteoporosis groups ($p = 0.03$). Likewise, RANKL levels differed between groups ($p < 0.05$) except between osteopenia and osteoporosis ($p = 0.14$). This shows the role of oxLDL and RANKL in bone mineral density.

Table 3. Distribution of RANKL levels with Bone Mineral Density

RANKL Levels (mU/L)	Bone Mineral Density (n=78)		
	Normal n=27	Osteopeni n=43	Osteoporosis n=8
≥ 11 (tinggi)	13(21,3%)	41(67,2%)	7 (11,5%)
< 11 (rendah)	14(82,4%)	2 (11,8%)	1 (5,8%)

In Table 3, it appears that higher RANKL levels are found more in osteopeni (95.3%) and osteoporosis (87.5%), but can also be found in normal bone mineral density (48.1%) whereas at lower RANKL levels more found in normal bone mineral density (15.4%).

DISCUSSION

This study aimed to see the relationship of RANKL to bone mineral density (DMT) in the group of women aged 30-60 years. The decline in bone mineral density that causes osteoporosis is becoming an increasingly important health problem along with the increasing elderly population.

1. Sample characteristics

This study consisted of 78 research subjects. The age range chosen as the sample is between 30-60 years with an average of 46.21 ± 7.80 years, the BMI range is 16-39.4 kg / m2 with an average of 24.71 ± 3.99 kg/m2, the age range of menopause 43 -54 years with an average of 50.00 ± 2.50 years. This age range was chosen with the consideration that the age of 30 years is associated with achieving peak bone mass (peak bone mass), if the peak bone mass is not achieved perfectly will have an impact on bone resistance in the future.

Table 1 results of this study indicate that a decrease in bone mineral density (osteopeni) has begun to occur in the young age group (30-44 years) even in all age groups, where in the age group 30-44 years (32.6%), age 45-51 years (32.6%) and age 52-60 years (34.8%). Osteoporosis all occurs in the age group 52-60 years.

The age group 52-60 years still has 3 samples (11.1%) with normal DMT, because this sample has a BMI> 30 kg / m^2 (obesity) and has undergone menopause> 3 years. There is an opinion stating that more weight will have a positive effect on DMT in postmenopausal women because it will stimulate bone formation by converting androgens to estrogen. Ovary is the main producer of estrogen (estradiol), besides it can also be produced by fat cells in the form of estron, these 3 samples are obese and postmenopausal which means excessive fat cells will produce enough estrogen for function bone remodeling. Excessive weight can also cause bones that support weight (vertebra and femur) will always get mechanical stress/ pressure from the muscles that make attachment around the bone periosteum. Periosteum is a highly cellular and vascular structure, rich in osteoblasts and nutrients so active differentiated osteoblasts for bone growth and repair.

Increased RANKL levels are not always followed by a decrease in DMT. This can be seen in Table 3 which shows that high RANKL levels are found in the osteopeni group (95.3%) and osteoporosis (87.5%), but can also be found in normal DMT (48.1%), but the Chi-Test square showed a significant relationship between the three DMT groups (p = 0,000, Table 3). This shows that high levels of RANKL affect the decrease in bone mineral density.

At high RANKL levels with normal DMT, it can be explained that in addition to RANKL, osteoclastogenesis is also influenced by several factors including, osteoprotogerin (OPG) and Macrophage Colony-Stimulating Factor (M-CSF), it can be explained that monocyte activity in atherosclerotic plaques can affect bone mineral density through the secretion of OPG, so that an increased OPG / RANKL ratio will inhibit the process of osteoclastogenesis. Besides this this condition can also be caused by the disruption of osteoclast maturation from osteoclast precursors due to interference with transcription factor signaling through molecular / protein adapters so that the maturation of osteroclasts in the osteoclastogenesis process is inhibited.

The activity and differentiation of osteoclasts is also mediated by interactions of molecules produced by osteoblasts namely OPG and RANKL, these two components have the opposite effect in bone remodeling. The Activator of Nuclear Factor kappa B ligand (RANKL) receptor will bind RANK to osteoclasts for osteoclast activation and differentiation, whereas OPG acts as a feed receptor by inhibiting RANKL binding to RANK receptors which will inhibit osteoclast differentiation, so that the RANKL / OPG ratio is very important in osteoclast differentiation which plays a role in bone regulation in maintaining the balance of bone remodeling, besides that Macrophage Colony-Stimulating Factor (M-CSF) also plays a role in osteoclastogenesis which is binding to its receptor (c-Fms) in osteoclast precursors.

CONCLUSION

From the results of the data analysis this research was obtained:

The Activator of Nuclear Factor kappa β Ligand (RANKL) receptor has a significantly negative correlation with bone mineral density. Increased levels of RANKL can result in a decrease in bone mineral density.

REFERENCES

1. Blair JM, Zhou H, Seibel MJ, Dunstan CR: *Mechanisms of disease: roles of OPG, RANKL and RANK in the pathophysiology of skeletal metastasis*. Nature Review Clinical Oncology 3, 2006, 41-49.
2. Boyle WJ, Simonet WS, *Lacey DL. Osteoclast differentiation and activation*. Nature 2003;423:337-342.
3. Colon EC, Lyles KW, Levine DA. *Prevalensi and predictor of osteoporosis Treatment of nursing home resident with known osteoporosis or recent fracture*. 2007, 18 (4): 553-559.
4. Ginaldi L, Benedetto M, Martinis M. *Osteoporosis, inflammation and ageing Immunity*. 2005, 2 : 14.
5. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V, Weaver C. *Peak bone mass.* Osteoporos Int, 2000, 11: 985-1009.
6. Johnell O, Kanis JA, Oden A, Sernbo I, Redlund-Johnell I, Petterson C, De Laet C, Jonsson B. *Fracture risk following an osteoporotic fracture*. Osteoporos Int, 2004, 15: 175-179
7. Lindsay R, CosmanF. *Osteoporosis* in Harrison"s,sprinciple of Internal Medicine. 2007, 16 th ed., 2268-79
8. Maziere C, Morlier P, Massyz. *Oxidized low-density lipoprotein elicits an intracellular calcium rise and increases the binding activity of the transcription factor NFAT*. 2005, 38; 472-480.
9. Maziere C, Savitsky V, Galmiche A, Gomila C, Massy Z. *Oxidized low density lipoprotein decreases Rankl-induced differentiation of osteoclasts by inhibition of Rankl signaling*. 2009, 221 (3) : 572-8.
10. McClung M. Role of RANKL *inhibition in osteoporosis* Arthritis Research and Therapy, 2007, 9(Suppl 1):S3.

11. Müller RJ and Richards RG. *Immunohistological Identification of The Osteoclast Formation Regulator Osteoprotogerin Ligan (OPGL/RANKL/TRANCE) in Human Bone Tissue*. European Cells and Materials. 003, 5 (2): 42-43.
12. McFarlane SI, Muniyappa R, Shin JJ, BahtiyarG, Sowers JR. *Osteoporosis and cardiovascular disease: brittle bonesand boned arteries, is there a link*? Endocrine. 2004, 23: 1-10
13. Parhami F, Basseri B, Hwang J, Tintut Y,Demer LL. *High density lipoprotein regulates calcification of vascularcells.* Circ Res. 2002, 91(7): 570–76.
14. Raisz LG. *Pathogenesis of osteoporosis: concepts, conflicts, and prospects*. J Clin Invest. 2005, 115: 3318-3325.
15. Racci N. *Molecular biology of bone remodeling*. Miner Bone Metab. 2008, 5(1): 49-56.
16. Samy I, McFarlane, John JR. Shin, Bahtiyar G and Sowers JR. *Osteoporosis and cardiovascular disease Brittle bones and boned arteries, is there a link*, Review. Endo : 2004, 23:1
17. Schoppet M, Preissner KT, Hofbauer LC. *RANK ligand and osteoprotegerin paracrine regulators of bone metabolism and vascular function*. Arterioscler Thromb Vasc Biol. 2002, 22: 549-53.
18. Tanko L, Y.Z. Bagger and C. Christiansen. *Low bone mineral density in the hip as a marker of advanced atherosclerosis in elderly women*, Calcif. Tissue Int. 2003, 73: pp. 15-20.
19. Tintut Y, Morony S, Demer LL. *Hyperlipidemia promotes osteoclastic potential of bone marrow cells ex vivo*. Arterioscler Thromb Vasc Biol. 2004, 24(2): 6-10
20. Walsh MC, Kim N, Kadono Y. *Osteoimmunology: Interplay between immune system and bone metabolism*; 2006, 24: 33-63
21. World Health Organitation Report. *Prevention and management of osteoporosis:* report of a WHO scientific group. (WHO technical report)series; 2003, 92.
22. Wren TA, Kim PS, Janicka A, Sanchez M and Gilsanz V.*Timing of Peak Bone Mass: Discrepancies between CT and DXA*. The Journal of Clinical Endocrinology and Metabolism. 2007, Vol. 92, No. 3: 938-941.
23. Yogesh Hole et al 2019 J. Phys.: Conf. Ser. 1362 012121