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RESEARCH

INTERLEUKIN-8 RELATED WITH BONE MINERAL DENSITY

(Interleukin-8 terkait Kepadatan Mineral Tulang)

Yurdiansyah Latif, Uleng Bahrun, Ruland Pakasi

ABSTRAK

Osteoporosis merupakan salah satu penyebab cacat pada usia lanjut karena kebahayaan patah tulang yang disebabkannya. Mulai usia 50 tahun kemungkinan mengalami patah tulang bagi perempuan adalah 40%, sedangkan bagi laki-laki 13%. Angka prevalensi osteopeni di Indonesia sebesar 41,7% dan osteoporosis sebesar 10,3%. Hal ini berarti dua dari lima penduduk memiliki kebahayaan untuk terkena osteoporosis. Interleukin-8 diduga berperan dalam merangsang pembuatan Receptor Activator of NF KappaB Ligand (RANKL) mRNA di osteoblast yang mengikat reseptor RANK di osteoklast yang berperan dalam penurunan kepadatan mineral tulang. Kajian ini bertujuan untuk mengetahui kadar Interleukin-8 dan hubungannya dengan kepadatan mineral tulang yang normal, osteopenia dan osteoporosis secara penentuan. Penelitian dilakukan secara potong lintang selama masa waktu antara bulan Mei 2012−Mei 2013 menggunakan data primer pemeriksaan kadar Interleukin-8 dan kepadatan mineral tulang pada perempuan yang berusia antara 30−60 tahun di Makassar. Data dianalisis dan diolah dengan uji Anova. Kadar interleukin-8 lebih tinggi di densitas mineral tulang (DMT) osteoporosis dibandingkan dengan DMT yang normal dan osteopenia dengan kadar IL-8 pada DMT normal 48,72±12,81, osteopenia 55,68±13,75, osteoporosis 62,06±24,45. Hubungan antara IL-8 pada perempuan dengan DMT yang normal dibandingkan dengan osteoporosis memperoleh nilai p=0,03, perempuan dengan DMT normal dengan osteopenia p=0,51 dan perempuan osteopenia dengan osteoporosis p=0,62. Didasari telitian ini, dapat disimpulkan bahwa terdapat hubungan bermakna antara peningkatan kadar IL-8 dengan kepadatan mineral tulang yang berkurang di kelompok perempuan osteoporosis dibandingkan dengan kelompok DMT yang normal. Para peneliti berpendapat untuk meneliti lanjutan dengan memperhatikan ciri indeks masa tubuh di sampel penelitian.

***Kata kunci**: Interleukin-8, kepadatan mineral tulang, osteopenia, osteoporosis*

ABSTRACT

Osteoporosis is one of the causes of disability in elderly females because of the risk of fractures caused by it. Starting at age of 50, the probability of in females is 40%, whereas it is 13% in males. Osteopenic prevalence rate is about 41.7% in Indonesia and the prevalence of osteoporosis is 10.3%. This means that two out of five people are at risk for osteoporosis. Interleukin-8 plays a role in stimulating the synthesis of the Receptor Activator of NF kappa B ligand (RANKL) mRNA in osteoblasts that bind to the receptor RANK on osteoclasts that plays a role in the decline in Bone Mineral Density (BMD). The aim of the study was to know the levels of interleukin-8 and its relationship with normal bone mineral density, osteopenic and osteoporotic by determination. A cross-sectional study was conducted during the period between May 2012−May 2013 primary using the data of Interleukin-8 levels and bone density in females aged between 30-60 years in Makassar. The data were analyzed and processed using the Anova test. The Interleukin-8 levels were higher than normal in osteoporosis and osteopenic with IL-8 levels in normal BMD 48.72±12.81, 55.68±13.75 osteopenic, and osteoporosis 62.06±24.45. The correlation between IL-8 in females with normal BMD compared to osteoporosis was p=0:03, females with normal BMD and osteopenic was p=0:51 and reviews those (females) with osteopenic and osteoporosis p=0.62. Based on this study, it can be concluded that there were significant correlations between elevated levels of IL-8 by reduced bone mass density in females with osteoporosis compared to normal BMD. The researchers advise to follow-up studies to be performed with more attention to the characteristics of Body Mass Index (BMI).

Key words: Interleukin-8, bone mineral density, osteopenic, osteoporosis

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INTRODUCTION

The physiological changes that occur in human beings are natural processes. The case is the same for females, who, after entering the age of 40 years, will reach the final stage of the fertile period (climacteric period) or perimenopause. Perimenopause is the period between premenopausal and menopausal changes, characterized by irregular menstrual cycles.[1] The physiological changes that appear most frequently in females is perimenopause/menopause, i.e. defects is in the skeletal system such as osteoporosis.[1] Osteoporosis is a disease characterized by reduced bone mineral density and changes in the microarchitecture of bone tissue, resulting in decreased bone density and increased bone fragility, thus resulting in easily broken bones.[2] The diagnosis of osteoporosis was set based on history, physical examination and examination of bone mineral density. Bone mineral density can be measured using the *Dual Energy X-Ray Absorptiometry* (DXA) which can measure bone density as well as the central and edge of certain sections of bone throughout the body. This tool has a higher degree of accuracy and is the gold standard examination of the bone density. The measurement of bone density is usually expressed by the T-score, the number of standard deviations the patient's bone density varying from the mean bone in a variety of normal subjects with the same sex.[3]

Osteoporosis is one of the causes of suffering and disability in the elderly because the risk of fracture increases with age.[4] In 2003, WHO recorded that more than 75 million people in Europe, America and Japan have osteoporosis resulting in 2.3 million fracture cases.[2] One in three females and one in five males are at risk of suffering hip or back fractures when entering the age of 80 years. Starting at the age of 50, the probability of fracture is 40% on females and 13% on males.[4] The data analysis of the risk osteoporosis in 2005 conducted by the Center for Nutrition MOH and a nutrition company in 16 regions in Indonesia showed the prevalence of osteopenia (early osteoporosis) of 41.7% and 10.3% of osteoporosis. This means that two out of five citizens has a risk of suffering from osteoporosis.[2] Based on these data, the osteoporosis disease is not only a problem in Indonesia, but it has become a global problem that requires thorough attention. Therefore, it is necessary to know the pathomechanism of osteoporosis in order to support its diagnosis and management.

Osteoclasts and osteoblasts have an important role in osteoporosis due to their function in bone homeostasis. Lucia *et al*[5], in his research stated that the immune response plays an important role in osteoporosis, especially in the activation and induction of T lymphocytes that *generate Receptor Activator of NF kappaB Ligand* (RANKL). *Receptor Activator of NF kappaB Ligand,* which has been known as a mediator for the interaction of T lymphocytes, also stimulates the maturation and activation of bone resorption.[5] Gur *et al*[6], examined for possible associations between serum interleukin-8 (IL-8) in 76 post-menopausal females with osteoporosis, then compared it with cytokines in healthy females. The results of this study showed no increase in serum IL-8 in patients compared to the comparison group.[6]

Interleukin-8 is initially only known as a chemotactic factor for neutrophils. During the inflammatory response, IL-8 is released from the inflamed tissue into the blood which then stimulates neutrophil response. Currently, IL-8 is known as a major factor that stimulates bone destruction and does not merely act as a chemotactic factor. Interleukin-8 stimulates the manufacture of RANKL mRNA in osteoblasts, RANKL being a membrane receptor in osteoblast or stromal cells that bind the receptor RANK on osteoclasts that are instrumental in the destruction of bone or decrease in bone mineral density.[7]

The controversial research and epidemiological data that are lacking in Indonesia, especially in Makassar, the place of study of the relationship between content of IL-8 with decreased bone mineral density, encouraged researchers to determine whether increased content of IL-8 participates in bone destruction associated with decreased bone mineral density of a person. The results were expected to examine the scientific explanation of the levels of IL-8 on bone mineral density in females aged between 30−60 years old, hence can be a reference material to further investigations concerning pathomechanism of the decrease of bone mineral density.

METHODS

The cross-sectional study which was conducted during the period between May 2012–May 2013 by taking the primary data on females aged between 30−60 in Makassar. Samples were females between the ages 30−60 years old who came to the Dr. Wahidin Sudirohusodo Hospital and other educational network hospitals in Makassar. The samples of the study were taken from subjects who did not smoke, drink alcohol and use drugs (steroids, anticonvulsants, estrogen hormones and hormonal contraception), had never

experienced a broken bone before, did not suffer from joint disease (rheumatoid arthritis or SLE), had their test results of liver and kidney function within normal limits, also had normal fasting blood sugar levels, showed no signs of infection in a routine blood test, and willing to be the subject of research by signing a letter of consent.

The subjects who met the participation criteria then had their bone mineral density examined and grouped into normal BMD, osteopenia and osteoporosis in the same number of samples, then had venous blood drawn for routine hematological examination, ALT, AST, urea, creatinine and fasting blood glucose. Insufficient sample volume for analysis such as jaundice and those undergoing hemolysis were excluded from this study. The measurement DMT used *General Electric Lunar DPX NT* with methods of *Dual Energy X-Ray Absorptiometry* (DXA) and construed in accordance with the real benchmark using a T-score (normal: T-score> -1, osteopenia: T-score between -1 and -2.5 Osteoporosis: T-score <-2.5). The content IL-8 was measured by ELISA method using *Quantikine Human Interleukin-8* ELISA *kit* from *R & D System* and read using *microplate reader* at a wave length of 450nm with a reference value of 0-60 pg/mL. The data was analyzed and processed statistically with *Oneway Anova* test.

RESULTS AND DISCUSSION

During the time period of May 2012 to January 2013, 75 samples of research were obtained that met its standards and had passed the examination of bone mineral density and levels of IL-8.

The results indicated the age range examined in the three groups of DMT in the same age range between 30−60 years (see Table 1).

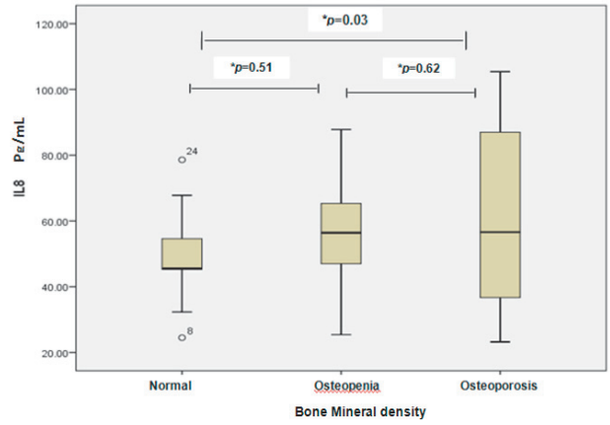

Figure 1. Level IL-8 in the normal bone mineral density, osteopenia and osteoporosis (*Oneway Anova Test*)

These results illustrated that the decrease in bone mineral density, not only in females who had menopause, such conclusions obtained in a previous study by Gur *et al*[6], but can also occur in females who had entered the age of 30.[6]

The levels of IL-8 occurred increasing as bone mineral density (Table 2) reduction. Results based on data levels interleukin-8 were found to be higher in osteoporosis DMT than in normal and osteopenic DMT.

The oneway Anova analysis indicated there was a significant association only in the group of females with normal BMD compared to osteoporosis group whose p=0.03 (Figure 1). Meanwhile, no significant relationship was found in the group of females with normal BMD compared with osteopenia (p=0.514) and osteopenic DMT compared with osteoporosis DMT (p=0.628). This was because the levels of IL-8 in both groups were still in the normal range levels IL-8.5

Table 1. Characteristics of the sample by age and bone mineral density

Variable	n (%)	Age range (year)
Group benchmark bone mineral density		
Normal	25 (33.3)	36-59
Osteopenia	25 (33.3)	30-54
Osteoporosis	25 (33.3)	32-60

Table 2. The relation between the level of Interleukin-8 with bone mineral density

Variable	Levels of IL-8 (pg/mL)		p*
	Average	SD	
Group benchmark bone mineral density			
Normal	48.72	12.81	
Osteopenia	55.68	13.75	.035
Osteoporosis	62.06	24.45	

* *Oneway Anova* Test

Immune and inflammatory factors play an important role in the pathophysiology of cardiovascular disease and osteoporosis. One such factor is the RANKL and osteoprotegerin (OPG).[8] Increased expression of RANKL in osteoclast activity and excessive formation may be triggered by an increase *IL-8* which led to a decrease in bone mineral.[9] *Receptor Activator of NF kappaB Ligand* and OPG are produced by osteoblast precursors. Osteoprotegerin is an inhibitor of osteoclastogenesis by binding to RANKL, making the RANKL not to bind to RANK on the osteoclast.[10,11] Osteoclastogenesis is regulated by the interaction between RANKL and RANK receptor.[12] RANKL/OPG system activates the formation, function and differentiation of osteoclasts involved in bone remodeling.[13]

Limitations of this study were the sample size that was still small, making the researchers unable to determine the *cut off* value which could be used to distinguish between normal and osteoporotic DMT.

CONCLUSION AND SUGGESTIONS

The increased levels of IL-8 in line with the decline in DMT, despite significant differences, was just proven between normal and osteoporosis DMT groups. This indicated a role IL-8 to the pathogenesis of osteoporosis. The researchers suggest further studies with a larger number of samples in order to determine the *cut off* value which can be used to distinguish between normal and osteoporotic DMT.

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