Research Report

Correlation between level of serum estrogen, c-telopeptide, and interleukin-6 in determining bone density in perimenopausal women

Hubungan kadar estrogen, c-telopoptide, dan interleukin-6 untuk menentukan densitas tulang pada perempuan perimenopause

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Abstract

Objective: To evaluate the role of estrogen as a menopausal biochemical marker to c-telopeptide (CTx) and IL-6 as a biochemical marker of mineral bone density in perimenopausal women.

Method: This is an observational, cross-sectional study in perimenopausal women between 45 - 51 years old, who matched the inclusion and exclusion criteria such as no contraception was taken before, no specific disease that could affect the laboratory findings. Samples were taken from outpatients clinic of Prof Dr. R.D. Kandou hospital. The patients were gathered for blood samples collection. Blood samples were tested in laboratory. All data were collected and being processed statistically using Pearson correlation coefficient (SPSS version 16).

Result: From 60 perimenopausal women (45 - 51 years old) sample, mean age was 47.98 ± 2.26 SD. Estrogen (estradiol) level: < 10 pg/ml, maximum 358 pg/ml, and mean 77.11 ± 86.2 SD. IL-6 level: minimum is 1.645 pg/ml, maximum 7.771 pg/ml, and mean 4.317 ± 2.112 SD. We found significant correlation between age and estrogen level (correlation level < 0.05) and CTx level (in level < 0.01). From regression curve, age has no significant correlation with IL-6 concentration. However, we found significant correlation between estradiol and CTx and IL-6 with respective correlation coefficient value of < 0.01. Likewise the correlation between CTx and IL-6 showed significant statistically with the value of the correlation coefficient < 0,01.

Conclusion: There is a significant correlation between estrogen, Il-6 and CTx. These variables play an important role in the occurrence of the physiological changes in the perimenopausal women. This study also showed the occurrence of a bone reabsorption process that was marked by the increase of CTx level in accordance with the increase in the age and the increase in the level of CTx together with the decline in the level of oestrogen. Therefore, prevention therapy of osteoporosis should begin in earlier age, before the further bone reabsorption take place.

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Keywords: c-telopeptide, bone mineral density, estrogen, interleukin-6, perimenopause women

Abstrak

***Tujuan**: Mengetahui peran estrogen sebagai penanda biokimiawi menopause terhadap c-telopeptide (CTx) dan IL-6 sebagai penanda biokimiawi penurunan densitas mineral tulang pada perempuan perimenopause.*

***Metode**: Penelitian ini adalah suatu studi observasional dengan pendekatan studi potong lintang (cross sectional) pada perempuan perimenopause usia 45 - 51 tahun yang memenuhi kriteria inklusi dan eksklusi. Faktor-faktor yang dapat mempengaruhi estrogen dan progesteron dikeluarkan dari sampel. Sampel diambil di Poliklinik RSUP Prof. Dr. R.D. Kandou Manado. Para pasien dikumpulkan pada hari tertentu untuk kemudian diperiksakan darahnya, pemeriksaan darah dilakukan di laboratorium swasta. Data yang ada dikumpul dan diolah secara statistik dengan menggunakan koefisien korelasi dari Pearson, dengan menggunakan program SPSS, versi 16.*

***Hasil**: Dari 60 sampel perempuan usia perimenopause (45 - 51 tahun) diperoleh mean usia sebesar 47,98 ± 2,26 SD. Kadar estrogen (estradiol) minimum diperoleh < 10 pg/ml sedangkan nilai maksimum sebesar 358 pg/ml dengan mean sebesar 77,11 ± 86,2 SD. Kadar CTx minimum diperoleh sebesar 0,03 μg/l, nilai maksimum sebesar 1,21 μg/l dengan mean sebesar 0,377 ± 0,226 SD. Kadar IL-6 minimum sebesar 1,645 pg/ml, nilai maksimum didapatkan sebesar 7,771 pg/ml dengan mean sebesar 4,317 ± 2,112 SD. Ditemukan suatu korelasi signifikan antara usia dengan kadar estrogen (pada level korelasi < 0,05) dan dengan kadar CTx (pada level < 0,01), dari kurva regresi, usia tidak memiliki korelasi signifikan terhadap konsentrasi IL-6. Namun korelasi signifikan antara estradiol dengan CTx dan IL6 dengan nilai koefisien korelasi masing-masing sebesar < 0,01. Demikian juga korelasi antara CTx dengan IL-6 menunjukkan signifikan secara statistik dengan nilai koefisien korelasi < 0,01.*

***Kesimpulan**: Terdapat suatu korelasi signifikan antara Estrogen, IL-6 dan CTx di mana variabel ini dianggap saling berperan dalam terjadinya perubahan fisiologis pada perempuan perimenopause. Studi ini juga menunjukkan telah terjadinya suatu proses reabsorbsi tulang yang ditandai dengan meningkatnya kadar CTx sesuai dengan pertambahan usia dan meningkatnya kadar CTx seiring dengan menurunnya kadar estrogen. Dengan demikian, terapi pencegahan osteoporosis dapat dimulai pada usia ini, sebelum terjadi proses reabsorbsi tulang lebih jauh.*

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***Kata kunci**: c-telopeptide, densitas mineral tulang, estrogen, interleukin-6, perempuan perimenopause*

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INTRODUCTION

One of the impacts of the improved health development in Indonesia is the increase of life expectancy, from 64.71 years (1995 - 2000) to 67.68 years (2000 - 2005), and it is estimated that elderly proportion in 2005 will be of 8.4% or 18.4 million people. This conditions, makes Indonesia be the fourth country in

the world after China, India, and United States with elderly population. Thus, Indonesia will face some degeneration problems such as perimenopausal women (45 - 51 years old), e.g. osteoporosis.[1] Perimenopause is a moment of which goes in the direction of menopause with normal ovarial cycle until complete end of menstruation.[2] This periode can be identified from the beginning of irregular menstrual period until the total menstruation cessation.

One problem take place in perimenopausal women is the decrease of bone mineral density as the result of decrease in estrogen level. Analysis held by the Nutrition Experimental and Development Center in Department of Health in 14 provinces showed that osteoporosis problem in Indonesia has achieved a caution level, which is 19.7%. The incidence of osteoporosis in Indonesia is 6 times higher than in Netherland. Five provinces with the highest risk of osteoporosis were South Sulawesi (27.7%), Middle Java (24.02%), Yogyakarta (23.5%), North Sumatera (22.82%), East Java (21.42%) and East Kalimantan (10.5%). Another study in Jakarta, Bandung, Semarang, Surabaya, and Medan in 2002 also showed that osteoporosis in Indonesia should have been cautioned. Of 101.161 respondents, there were 29% (include perimenopausal and menopausal women) have suffered from osteoporosis.[3]

Regarding the needs of early prevention of the decrease in bone mineral density, researchers think that it is necessary to research about estrogen role as biochemical marker in menopause against c-telopeptide (CTx) and IL-6 as the biomechanical marker of bone mineral density in perimenopausal women.

Decrease of bone mineral density denotes a chronic continuous processes that leads to osteoporosis. Decrease of bone mineral density in perimenopausal ages result in high fragility fracture rate in this group of age. Examination with DEXA is a gold standard to determine the diagnosis of osteoporosis. The clinical fact shows that osteoporosis is often diagnosed after fracture. Subsequently, there is a necessity of a dependable biomarker and individual susceptibility to experience osteoporosis.[4]

Estrogen level could be used as a biomarker from the beginning of perimenopausal period. Perimenopause will started when the estrogen level reach 108 pg/ml and in the late perimenopause the estrogen level reach 48 pg/ml.[5-7] Estrogen influence bone resorption through estrogen receptor in osteoclast and osteoblast.[8] Hipoestrogenic environment such as in perimenopause period will decrease calcium absorbtion in the bowel.[9] Consequently, in the perimenopause period, series of bone catabolic activity that leads to osteoporosis will take place. Estrogen influences calcium absorption from the bowel and excretion through kidney. Its other main benefit is blockage of bone calcium resorption. Within the premenopausal period, estrogen maintains bone mass through blocking the activity of parathyroid hormone (PTH) and increasing calcitonin.[10]

Estrogen deficiency results in the increase of proinflammatory cytokine production in the bone microenvironment. Those cytokine are: IL-1, IL-6, TNFα, GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor), and Prostaglandine E2. Those cytokine acts through increasing pool of proosteoclast inside the bone marrow.[11] IL-6 was the chosen cytokine in this experiment as in vitro experiment shows that IL-6 has a positive role in osteoclastogenesis.[12,13] Bone degradation marker is a C-telopeptide and N-telopeptide, whereas bone matrix production marker is alkali phosphatase.[14] In the past, alkali phosphatase was used to identify osteoblast activity, whereas bone resorption marker is hidroxyproline. The better understanding then shows that both parameters have low sensitivity and specificity.[15] Compared to other markers, CTx has a better sensitivity and more accurate result in measurement of serum(13). C-telopeptide[16,17], IL-6[4,18], are biomarkers that have effect in bone mineral density. Those factors make new hope that osteoporosis in the future could be predicted earlier before significant decrease of bone mineral density and fracture occur. Fracture in osteoporosis patients need more time to heal and decrease patient's quality of life.[19,20]

Relationship between serum estrogen concentration and C-telopeptide (CTx) and relationship between serum estrogen concentration and IL-6 need to be examined. Estrogen as a biomarker against perimenopause, whereas CTx and IL-6 as biomarkers of bone catabolism might be influence each other. If there is a correlation, we look forward for a further study that could be done to find whether CTx and IL-6 level could be used as a predictor of decrease in bone mineral density in the future.

METHOD

This is a cross sectional observational study in perimenopausal women, age of 45 - 51 years who meet inclusion criteria. Data were analyzed to see the motive-consequence relationship (correlation). Study population is perimenopausal women present in the outward obstetric-gynecologic clinic Prof. dr. R.D. Kandou Manado General Hospital. Study sample is component of study population who meets the inclusion and exclusion criteria.

1. Inclusion criteria are:
   Perimenopausal women 45 - 51 years old, sign the informed consent to participate in this study, clinically healthy, except problems of bone tenderness without certain disease, free from hormonal contraception for the last 6 months, BMI within normal range, never had fracture before experiment, non-smoker (active and passive).
2. Exclusion criteria:
   Lysis or damage sample through examination process, factors listed in Table 1, consumption of certain drugs which will affect bone metabolism 21: glucocorticoids, cyclosporine, cytotoxic drug, anticonvulsant, alcohol, aromatase inhibitor, thyroxine, aluminum, GnRH agonist, heparin, lithium.

Several other factors despite estrogen hormone deficiency in perimenopausal period affect the risk of osteoporosis, subsequently those factors should be vanished away to prevent affecting experimental result. Those factors are listed in Table 1[21]:

Table 1. Factors affecting bone mineral density other than estrogen deficiency in menopausal period.[21]

Hypogonadal states	Hematologic disorders/ malignancy
Turner syndrome	Multiple myeloma
Klinefelter syndrome	Lymphoma and leukemia
Anorexia nervosa Hypothalamic amenorrhea	Malignancy-associated parathyroid hormone (PTHrP) production
Hyperprolactinemia	Mastocytosis
Other primary or secondary hypogonadal states	Hemophilia
Thalassemia	Selected inherited disorders
Endocrine disorders	Osteogenesis imperfecta
Cushing's syndrome	Marfan syndrome
Hyperparathyroidism	Hemochromatosis
Thyrotoxicosis	Hypophosphatasia
Type 1 diabetes mellitus	Glycogen storage Diseases
Acromegaly	Homocystinuria
Adrenal insufficiency	Porphyria
Nutritional and gastrointestinal disorders	Ehlers-Danlos Syndrome
Malnutrion	Menkes' syndrome
Parenteral nutrition	Epidermolysis bullosa
Malabsorption syndromes	Other disorders
Gastrectomy	Immobilization
Severe liver disease, especially biliary cirrhosis	Chronic obstructive pulmonary disease
Pernicious anemia	Pregnancy and lactation
Rheumatologic disorders	Scoliosis
Rheumatoid arthritis	Multiple sclerosis
Ankylosing spondylitis	Sarcoidosis
	Amyloidosis

Correlation test was done by Pearson test to determine the correlation between estrogen and CTx and IL-6.

RESULTS

During study period, 60 perimenopausal women were obtained. Each sample was evaluated for estradiol, CTx, and IL-6 level and analyzed statistically.

Sample Characteristics

Of 60 perimenopausal women samples (45 - 51 years old), the age mean was 47.98 ± 2.26 SD. Minimum estrogen (estradiol) level was 10 pg/ml, whereas maximum estrogen level was 368 pg/ml with mean of 77.11 ± 86.2 SD. Minimum CTx level was 0.03 μg/l, maximum level was 1.21 μg/l, with mean of 0.377 ± 0.226 SD. Minimum IL-6 level was 1.645 pg/ml, maximum level was 7.771 pg/ml, with mean of 4.317 ± 2.112 SD.

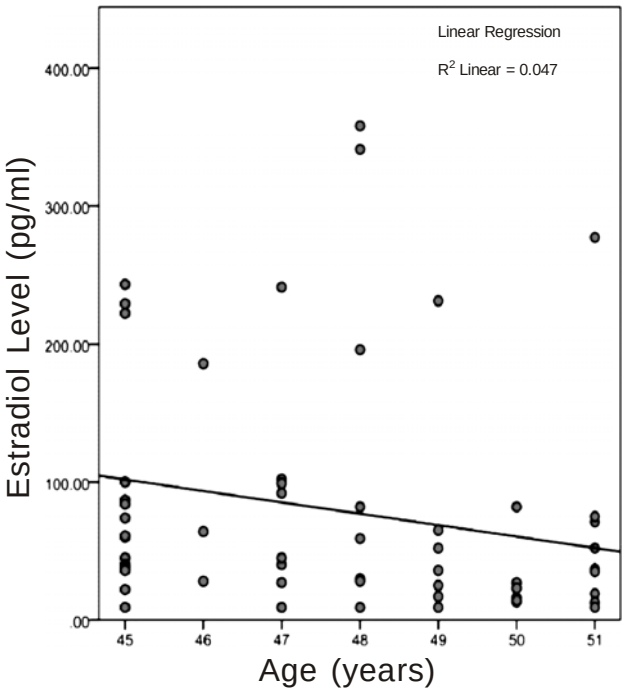

Figure 1. Correlation between age and estrogen concentration

There is a significant correlation between maternal age and estradiol serum concentration. The estradiol level tend to decrease as the maternal age advance (Figure 1). From the statistical analysis, we found there is no correlation between maternal age and IL-6 serum concentration. This finding is not in accordance with the theory that state IL-6 serum would increase as the maternal age advanced.

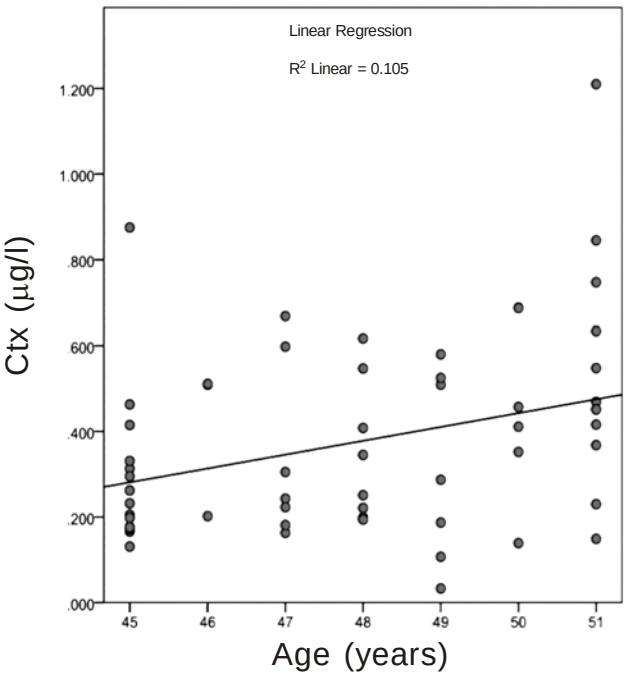

Figure 2. Correlation between age and CTx

There is a significant correlation between maternal age and CTx serum concentration. The CTx level tend to increase as maternal age advanced (Figure 2).

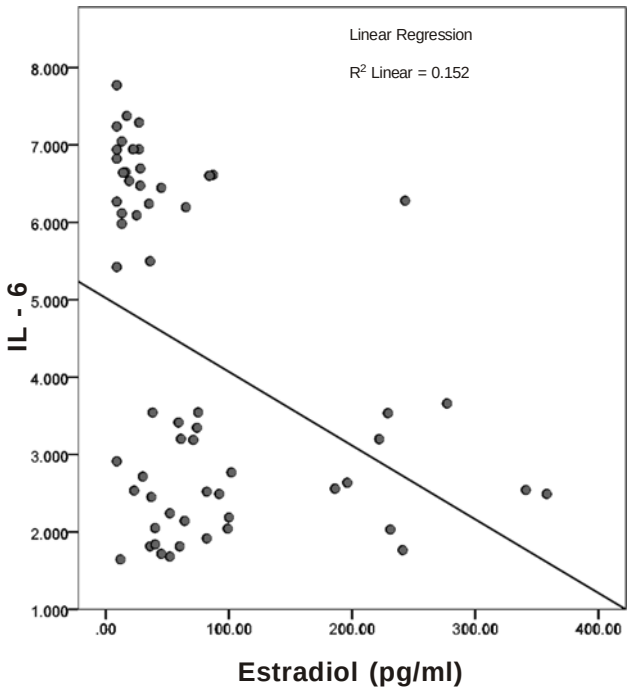

Figure 3. Correlation between Estradiol and IL-6

There is a signifficant correlation between the estradiol serum and IL-6 serum concentration. From the linear curve we could concluded that as the serum estradiol concentration increase, the IL-6 concentration decrease. (Figure 3)

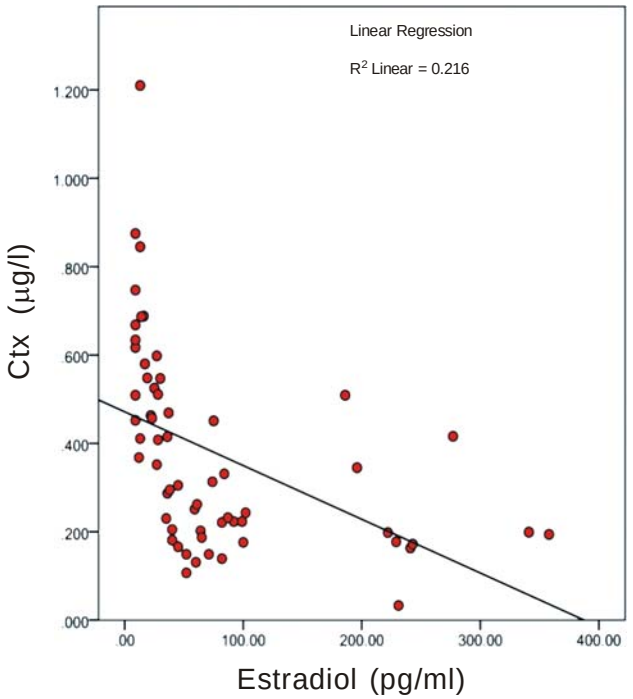

Figure 4. Correlation between Estradiol and CTx

As the linear curve between estradiol and IL-6, the linear curve between estradiol and CTx also show a same correlation, decrease of CTx concentration as the estradiol concentration decrease. (Figure 5)

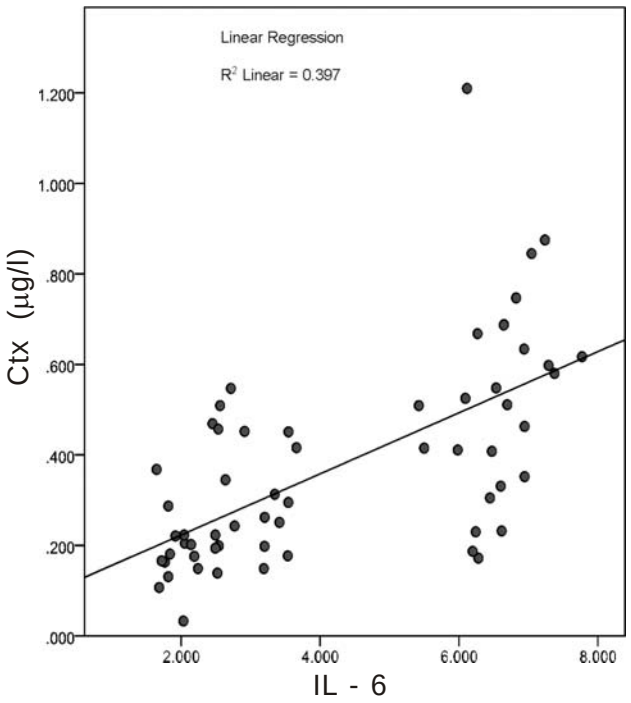

Figure 5. Correlation between IL-6 and CTx

DISCUSSION

Statistic data analysis found a significant correlation between age and estrogen level (correlation level < 0.05) and with CTx level (level < 0.01). This is in accordance with the theory that elderly population will have ovarian function decreased. Thus, bone remodelling process will be shifted to negative direction. Further data analysis shows that age does not have significant correlation with IL-6 concentration. The reason is so many factors affecting IL-6 level in blood, such as immunity and. Correlation of age and IL-6 in this experiment was contradictive with Xu et al (2003) study which stated that the more increase of age, the higher serum IL-6 level and results in it the more bone mass decrease. Other potential cause of the difference between this study and Xu et al is because Xu's experiment was done in women with age older than 50 years old, so the more significant correlation was drawn, compared to this experiment, with younger age range (45 to 51 years old).

Further statistical analysis shows significant correlation between estradiol with CTx and IL-6 with coefficient correlation each of < 0.01. The correlation between CTx and IL-6 also shows statistically significant coefficient correlation of < 0.01. To observe those correlations more clearly, we try to describe the correlation through regression curve.

It is shown that relationship between age and estrogen (estradiol) level build a linear curve with coefficient regression (R^2 = .047), also correlation between age and CTx level has R^2 = .105. Correlation between age and IL-6 also build linear curve with R^2 = .012, though their correlation was not significant statistically. Correlation between estrogen level with CTx and IL-6 build a linear curve with coefficient regression each of R^2 = .216 and R^2 = .152. The last was correlation between IL-6 and CTx, a linear curve was built with R^2 = .397.

The limitation of this study is that the bone mineral density was not examined because of limited facility in our centre. DMT examination should be done using DEXA (Dual Energy X-ray Assay) which is a gold standard for DMT examination. DMT examination was needed to evaluate to which extent the bone resorption process take place, and to confirm laboratory examination results.

CONCLUSION

There was a significant correlation between estrogen, IL-6 and CTx, the variable of which act each other in occurrence of physiologic changes of perimenopause women. Subsequently, osteoporosis prevention therapy should be started in this age, before further bone reabsorption process take place.

Further study to compare estrogen, IL-6, and CTx level in bone mineral density using DEXA in perimenopausal women was needed to further observe the perimenopause impact in those women.

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