

Correlation Between 25-Hydroxyvitamin D and Estradiol Serum Level in Determining Bone Density in Menopausal Women

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Abstract— Postmenopausal osteoporosis is one of the world's concern with high morbidity and mortality which is marked by low bone density which makes the bone fragile and increases the risk of fracture. It had be known that estrogen plays a role in women's bone health, that is to maintain the activity of osteoblasts and osteoclasts. Vitamin D which is reflected by 25 Hydroxyvitamin D (25(OH)D) serum is also known to be important to stimulate the absorbtion of calcium in the small intestine for bone mineralization and in the state of vitamin D deficiency, it increases bone turnover by causing secondary hyperparathyroidism. To know the correlation between 25(OH)D and estradiole serum level with bone densities in menopausal women. This is an analytic research with cross sectional approach performed on 36 menopausal midwives/nurses in RSUP. H. Adam Malik Medan, from September-October 2015. We measure the level of 25(OH)D and estradiole serum, bone density were measured using Dual energy X-ray Absorbtiometry (DXA) method, and then were analyzed with multiple correlation test. From the characteristics, it is found that most of them is aged > 50 (80,6%), menopause since 3-4 years ago (55,6%), normoweight BMI (47,2%), 25(OH)D level ≤ 20 ng/ml (69,4%), estradiole level ≤ 20 pg/ml (88,9%), and osteopenia (63,9%). Based on chi-square, from the risk factors, only Body Mass Index shows a significant correlation with bone density and estradiole rate ($p=0,015$ and $p=0,005$). According to Spearman correlation test, there is no significant corelations between 25(OH)D serum level and T-score ($r = 0,261$, $p>0,05$). There is a significant positive corelations between estradiole serum and T-score ($r=0,53$, $p<0,05$) and with multiple corelation test, there is a significant positive corelations between 25(OH)D rate and estradiole serum with T-score ($R=0,0663$ and $p<0,05$). Level of 25(OH)D and Estradiole serum have a contribution value as much as 44% towards bone density ($R^2=0,44$). There is a significant positive corelations between the level of 25(OH)D and estradiole serum towards bone density score (T-score).

Keywords— 25 Hydroxyvitamin D, estradiole, osteoporosis, T-score, bone mineral density, menopause.

I. INTRODUCTION

One of the impacts of improved health development in Indonesia is the increasing life expectancy of 64-71 years old (1995-2000) to 67-68 years old (2000-2005). This makes Indonesia became the fourth country in the world after China, India, and the United States with the elderly

population. Thus, there will be an increase in the problem of degeneration such as in menopause is osteoporosis [1].

The pathogenesis of postmenopausal osteoporosis involves many factors such as estrogen deficiency, low calcium intake, vitamin D deficiency and secondary hyperparathyroidism. Serum estradiol levels were decreased to a loss of markers of ovarian function in postmenopausal women. It is known that estrogen plays an important role in determining bone health in women, ie in maintaining a balance of work osteoblast (bone formation) and osteoclasts (bone resorption).

Hipoestrogen circumstances in postmenopausal women at increased risk of osteoporosis with an increased osteoclast formation and increased bone turnover [2], [3], [4].

The majority of studies indicate no association between serum estradiol level with Bone Mineral Density (BMD). Pham et al. (2013) showed a significant correlation between the levels of estradiol with BMD in both men and women. Hulking et al. (2004) showed estradiol significantly associated with lumbar vertebra, and only a few relationships in the proximal femur and total order. Mawi et al. (2010) showed no relationship between the levels of estradiol with femoral neck BMD but not at the lumbar spine and distal radius. Rogers et al. (2012) showed serum estradiol levels were positively associated with bone mass density absolute in all bodies [5], [6], [7].

The most important role of vitamin D is to trigger the absorption of calcium in the small intestine to the function of bone mineralization, without the help of vitamin D, only 10-15% of calcium and 60% phosphate can be absorbed in the intestine, compared with the aid of vitamin D which increase

the absorption of calcium to 30 -40% and 80% phosphate. Vitamin D deficiency, triggers the secretion of parathyroid hormone, which if sustained will increase osteoclast formation which will dissolve the collagen matrix of bone demineralization and occurs so that the calcium ions released into circulation [8], [9], [10].

There is still a controversial relationship between the levels of 25 (OH) D serum and bone density. Some studies show different results of research that indicates there is a relationship between vitamin D levels with bone density while Rassouli et al research and studies did not show an association between vitamin D levels with bone density as well as in the research of Allali Garnero et al and Hosseinpanah et al [5], [6], [7]. Based on the various results of this study, researchers are interested in studying the relationship between the levels of 25 (OH) D as a measure of a store of vitamin D in the human body and serum estradiol with bone density in postmenopausal women using dual energy X-ray Absorbtiometry (DXA) as the gold standard for osteoporosis examination.

II. METHOD

This study was approved by the Ethics Committee of the Faculty of Medicine, University of North Sumatra. All study participants were included in the study, were given an explanation of the purpose, benefits, and risks of research and the responsibilities of researchers. After the participants understood, they were required to give an approval by signing a statement of consent that had been provided. Every patient was entitled to know the results of the inspection and may withdraw from the study if they were not willing to continue the research.

A total of 36 samples consisting of menopausal midwives and nurses in Dr H. Adam Malik were included in the study which was carried out from September 2015 - October 2015.

Inclusion criteria for the study was a midwife and nurse in RSUP. HAM hospital and other hospital who menopausal / no menstrual ≥ 12 consecutive months and are willing to participate in the study and signed the form of availability. While exclusion criteria are: broken blood serum; withdrawal from the study; suffering from malignant disease, kidney

disease, parathyroid disease, liver disease; use of hormone replacement therapy; had a history of surgical removal of the ovaries; and the habit of smoking and drinking alcohol.

The study participants then had their blood drawn as much as 10cc of median cubital vein participants to measure levels of estradiol and 25 (OH) D, which is examined in the laboratory Prodia. After that patients were welcome to follow the procedure BMD measurements by DXA method in RS. Medan Setia Budi.

Levels of vitamin D in this study were defined as levels of 25 (OH) D in the serum were measured using ELISA and then categorized into category 1 (≥ 20 ng / ml) and 2 (< 20 ng / ml) and using the measuring scale ratio. Estradiol levels in serum were measured by CLIA and also classified into two categories, namely the category 1 (> 20 pg / ml) and the category 2 (≤ 20 pg / ml) with a measuring scale ratio.

Bone mass density is defined as bone mineral content which is measured at the femoral neck bone by using DXA (Dual energy X-ray Absorbtiometry) and categorized into category 1 (Normal values ≥ -1), category 2 (Osteopenia with values < -1 s / d - 2.5) and category 3 (Osteoporosis with a score of < -2.5) with ordinal measuring scale.

Data were collected and analyzed statistically to see the frequency distribution variables studied. Then analyzed to relate the levels of 25 (OH) D serum and serum estradiol on bone density.

III. RESULT

Characteristics of research subjects are described in Table 1 and it was found that the age of most research subjects is > 50 years old (80.6%), with long menopause is generally 3-4 years (55.6%) with a body mass index normal weight (47.2%) , levels of 25 (OH) D serum most common with the results of deficiency (< 20 ng / ml), ie 69.4%, estradiol levels are often found ≤ 20 pg / ml which was 88.9% while the highest bone density status characteristics found in osteopenia group (63, 9%).

Based on table 4.1. it can be seen that based on the characteristics of the age of the study most subjects were from the age group > 50 years as many as 29 people (80.6%) with menopause generally 3-4 years old were 20 people (55.6%),

while for the characteristics of body mass index (IMT) are largely normoweight many as 17 people (47.2%), the rest overweight and obese, may not find study subjects included underweight group.

TABLE I
CHARACTERISTICS OF RESEARCH SUBJECTS

| Characteristics | N | % |
|---------------------------------|----|------|
| Category Age | | |
| ≤ 50 yo | 7 | 19,4 |
| > 50 yo | 29 | 80,6 |
| Duration of Menopause | | |
| 1 - 2 yo | 6 | 16,7 |
| 3 - 4 yo | 20 | 55,5 |
| ≥ 5 yo | 10 | 27,8 |
| IMT | | |
| Underweight | 0 | 0 |
| Normoweight | 17 | 47,2 |
| Overweight | 10 | 27,8 |
| Obese | 9 | 25,0 |
| Level of 25(OH)D Serum | | |
| < 20 ng/ml | 25 | 69,4 |
| ≥ 20 ng/ml | 11 | 30,6 |
| Level of Estradiol Serum | | |
| ≤ 20 pg/ml | 32 | 88,9 |
| > 20 pg/ml | 4 | 11,1 |
| Bone density status | | |
| Normal | 7 | 19,4 |
| Osteopenia | 25 | 69,4 |
| Osteoporosis | 4 | 11,1 |

For levels of 25 (OH) D serum most common with the results of deficiency (<20 ng / ml) as many as 25 people (69.4%) and the rest ≥20ng / ml (11 /

30.6%), while the estradiol levels encountered ≤ 20pg / ml as many as 32 people (88.9%) and only 4 (11.1%) who had estradiol values above normal values (> 20pg / ml). Based on the characteristics of the most common bone density status in osteopenia group of 25 people (69.4%), 7 (19.4%) in the group of normal bone density and 4 (11.1%) in the group of osteoporosis.

Based on table 4.2. picture obtained the status of bone density by a factor of risk, to categories of age ≤ 50 years found most osteopenia as many as 6 people (85.7%) and only 1 (14.3%) who had normal bone status, without having encountered the subject of osteoporosis , At the age of > 50 years found most osteopenia as many as 19 people (65.5%) followed by normal bone density status as 6 people (20.7%) and osteoporosis as many as four people (13.8%).

To the old category menopause, between 1-2 years most have osteopenia 5 people (83.3%) and normal bone density by 1 person (16.7%) in the absence of the subject of osteoporosis. In the long span of 3-4 years of menopause was found most osteopenia as many as 14 people (70%), normal 5 people (25%) and osteoporosis 1 (5%). While the menopause ≥ 5 years old, most still in osteopenia 6 (60%), followed by osteoporosis 3 people (30%) and normal 1 person (10%).

TABLE II
STATUS OVERVIEW BASED RISK FACTORS BONE DENSITY

| Characteristics | Bone density | | | | | | P value |
|------------------------------|--------------|------|------------|------|--------------|------|---------|
| | Normal | | Osteopenia | | Osteoporosis | | |
| | n | % | n | % | n | % | |
| Category Age | | | | | | | |
| ≤ 50 yo | 1 | 14,3 | 6 | 85,7 | 0 | 0 | 0,822* |
| > 50 yo | 6 | 20,7 | 19 | 65,5 | 4 | 13,8 | |
| Duration of Menopause | | | | | | | |
| 1 - 2 yo | 1 | 16,7 | 5 | 83,3 | 0 | 0 | 0,368* |
| 3 - 4 yo | 5 | 25 | 14 | 70 | 1 | 5 | |
| ≥ 5 yo | 1 | 10 | 6 | 60 | 3 | 30 | |
| IMT | | | | | | | |
| Normoweight | 0 | 0 | 14 | 82,4 | 3 | 17,6 | 0,015* |
| Overweight | 2 | 20 | 7 | 70 | 1 | 10 | |
| Obese | 5 | 55,6 | 4 | 44,4 | 0 | 0 | |

*Fisher exact test

For the category of body mass index normal weight found most osteopenia as many as 14 people (82.4%), followed by osteoporosis as many as three people (17.6%) with no common status normal bone density. In the overweight group found most osteopenia of 7 people (70%), followed by normal bone density status 2 (20%) and osteoporosis 1 person (10%). In the obese group with the most common status normal bone density by 5 people (55.6%) and osteopenia as many as four people (44.4%), osteoporosis is not found in the obese group.

Statistically with Chi-square test showed no significant difference in bone density based on the characteristics of age, duration of menopause ($p >$

0.05) but showed significant differences between body mass index and bone density ($p = 0.015$).

Based on Table 4.3. picture obtained levels of 25 (OH) D serum is based on risk factors, to ≤ 50 years age category with the most common normal status of 4 people (57.1%) and in those aged > 50 years with the most common deficiency status as many as 22 people (75, 9%), with mean levels of 25 (OH) D in subjects aged ≤ 50 years (21.59 ± 4.61) is greater than the average subject age > 50 years (15.52 ± 5.33).

To the old category menopause, between 1-2 years obtained the same number of subjects between normal and deficient status.

TABLE III
OVERVIEW LEVELS OF 25 (OH) D SERUM BASED RISK FACTORS

| Characteristic | 25(OH)D serum level | | | | P value | Mean \pm SD (ng/ml) |
|-----------------------|---------------------|------|-----------------|------|---------|-----------------------|
| | < 20 ng/ml | | ≥ 20 ng/ml | | | |
| | n | % | n | % | | |
| Category Age | | | | | | |
| ≤ 50 yo | 3 | 42,9 | 4 | 57,1 | 0,213* | 21,59 \pm 4,61 |
| >50 yo | 22 | 75,9 | 7 | 24,1 | | 15,52 \pm 5,33 |
| Duration of Menopause | | | | | | |
| 1 - 2 yo | 3 | 50 | 3 | 50 | 0,541** | 19,7 \pm 6,26 |
| 3 - 4 yo | 15 | 75 | 5 | 25 | | 16,53 \pm 5,1 |
| ≥ 5 yo | 7 | 70 | 3 | 30 | | 15,19 \pm 6,47 |
| IMT | | | | | | |
| Normoweight | 14 | 82,4 | 3 | 17,6 | 1,0* | 16,39 \pm 4,01 |
| Overweight | 5 | 50 | 5 | 50 | | 16,79 \pm 8,58 |
| Obese | 6 | 66,7 | 3 | 33,3 | | 17,36 \pm 5,01 |

*Continuity correction test

**Fisher exact test

In the long span of 3-4 years of menopause was found by as many as 15 people deficiency status (75%) and at menopause ≥ 5 years old were found most also with deficiency status of 7 people (70%), but based on the average levels of 25 (OH) D serum, at menopause 1-2 years old group (19.7 ± 6.26) was higher than the 3-4 years group (16.53 ± 5.1) and ≥ 5 years group (15.19 ± 6.47).

For the category of body mass index with the highest encountered normoweight deficiency status as many as 14 people (82.4%), in the overweight

group found the same number of subjects between normal and deficient status, while the obese group with the most common deficiency status as many as 6 people (66, 7%). For the mean value of the levels of 25 (OH) D almost same three groups, namely: normoweight (16.39 ± 4.01), overweight (16.79 ± 8.58) and Obese (17.36 ± 5.01).

Statistically with Chi-square test showed no significant difference in levels of 25 (OH) D serum is based on the characteristics of age, duration of menopause or BMI ($p > 0.05$).

TABLE IV
SERUM ESTRADIOL LEVELS OVERVIEW BASED RISK FACTORS

| Characteristic | Estradiol serum level | | | | P value |
|-----------------------|-----------------------|------|------------|------|---------|
| | ≤ 20 pg/ml | | > 20 pg/ml | | |
| | N | % | N | % | |
| Category Age | | | | | |
| ≤ 50 yo | 7 | 100 | 0 | 0 | 0,710* |
| >50 yo | 25 | 86,2 | 4 | 13,8 | |
| Duration of Menopause | | | | | |
| 1 - 2 yo | 5 | 83,3 | 1 | 16,7 | 1,0** |
| 3 - 4 yo | 18 | 90 | 2 | 10 | |
| ≥ 5 yo | 9 | 90 | 1 | 10 | |
| IMT | | | | | |
| Normoweight | 17 | 100 | 0 | 0 | 0,005* |
| Overweight | 10 | 100 | 0 | 0 | |
| Obese | 5 | 55,6 | 4 | 44,4 | |

*Uji continuity correction

**Fisher exact test

According to the table 4.4. picture obtained serum estradiol levels based on risk factors, for the category of age ≤ 50 years found all subjects had higher levels of ≤ 20 pg / ml, at age > 50 years was found with the highest levels of ≤ 20 pg / ml as many as 25 people (86.2%).

For all the old category menopause, most subjects were found with levels of ≤ 20 pg / ml, namely; on a long menopause 1-2 years as many as five people (83.3%), on a long menopause 3-4 years as many as 18 people (90%) and the menopause ≥ 5 years old were 9 people (90%).

TABLE V
THE RESULTS OF CORRELATION TEST LEVELS OF 25 (OH) D SERUM WITH A SCORE OF BONE DENSITY

| Research variable | R value | P value |
|-------------------------|---------|---------|
| Serum level of 25 (OH)D | 0,261 | 0,125 |
| T-Skor | | |

For the category of body mass index and overweight normoweight found all subjects with high levels of ≤ 20 pg / ml, while the obese group were also found with the highest levels of ≤ 20 pg / ml as many as five people (55.6%).

Statistically with Chi-square test showed no significant differences in serum estradiol levels based on the characteristics of old age and menopause (p > 0.05) but there are significant differences in serum estradiol levels based on body mass index (p = 0.005).

Based on the Spearman correlation test between serum levels 25 (OH) D with T-score, it was found that there is no significant correlation, with correlation value r=0,261 and p>0,05.

TABLE VI
THE RESULTS OF CORRELATION TEST LEVELS OF 25 (OH) D SERUM WITH A SCORE OF BONE DENSITY.

| Research variable | R value | P value |
|--------------------------|---------|---------|
| Serum level of estradiol | 0,530 | 0,001 |
| T-Skor | | |

Based on Spearman correlation test between the serum levels of estradiol with T-scores showed that there is a significant positive correlation, with the strength of the correlation r = 0.53 and p < 0.05. This shows the level of correlation between the serum levels of estradiol were with T-scores.

TABLE VII
THE RESULTS OF CORRELATION TEST LEVELS OF 25 (OH) D AND SERUM ESTRADIOL ON BONE DENSITY SCORE.

| Research Variable | R value | R Square | P value |
|--------------------------|---------|----------|---------|
| Serum level of 25(OH)D | 0,66 | 0,44 | 0,00 |
| Serum level of estradiol | 3 | | |
| T-Skor | | | |

Based on the test results of double correlation between the levels of 25 (OH) D and estradiol serum bone density score, it is known that the magnitude of the correlation levels of 25 (OH) D and serum estradiol levels (simultaneously) to bone density score was calculated by the correlation coefficient is 0.663, and p < 0.05 it demonstrates the strength of correlation of the moderate category and thus the hypothesis that there is a significant correlation between the levels of 25 (OH) D and serum estradiol with bone density of postmenopausal women received.

With the value of R square = 0.44, it can be concluded that the contribution levels of 25 (OH) D and serum estradiol on bone density score is 44%.

Based on Spearman correlation test between serum levels of 25 (OH) D with T-scores showed that there was no significant correlation (Table 2), with the strength of the correlation $r = 0.261$ and $p > 0.05$. Thus the hypothesis that there is a significant correlation between 25 (OH) D serum level of postmenopausal women with bone density declined.

IV. DISCUSSION

Menopause is a physiological process that begins with the decline of reproductive function and hormonal changes that followed the decline of various organ function, including reduction of estrogen, the results of this study (Table 4.4) obtained serum estradiol levels ≤ 20 pg / ml to 88.9% of all subjects, as well as if the review is based on the characteristics of age, duration of menopause and body mass index and overweight normoweight showed 80-100%, if found 4 study subjects with higher levels can be caused by various factors such as the source of estradiol derived from ekstrasglandular indicated by the index obese body mass [11], [12].

Based on this study, it was found postmenopausal women with low bone density values, only 19.4% with normal bone density, and only 4 (11.1%) who have osteoporosis, seen from the fourth characteristic of this subject, entirely included in the category of obese and with higher estradiol levels, these results prove the relationship of body mass index on bone density [13], [14].

It was also found a decrease in the absorption of vitamin D function in the gut and decreasing levels of 7-dehydrocholesterol in the epidermis which is a provitamin D and therefore contributes to declining reserves of vitamin D for older women, the results of this study obtained 69.4% of menopausal women deficient in vitamin D with a cut off point of 20 ng / ml (table 4.1.). Several studies determined number 30 ng / ml as the cut off point, referring to this value, the entire subject of research at the level of vitamin D deficiency by age category, it found levels of 25 (OH) D serum of women aged ≤ 50 years (21.59 ± 4.61) showed higher values than in older women (15.52 ± 5.33).

A meta-analysis has shown varying figures for the prevalence levels of 25 (OH) D serum < 20 ng / mL (50nmol / L) ranging from 1.6 to 86% of

postmenopausal women who stay at nursing house [12]. international epidemiological studies recently reported approximately 64% of postmenopausal women with osteoporosis had serum 25 (OH) D < 30 ng / mL (< 75 nmol / L) or 30.8% when vitamin D threshold was lowered to 20 ng / mL (50nmol / L) throughout the world. This is a large study involving 2,606 postmenopausal women with osteoporosis in 18 countries. The prevalence of deficiency (< 30 ng / ml) of vitamin D in equatorial countries, represented by Brazil, Mexico, Malaysia, and Thailand, respectively 42.4%, 67.1%, 48.7% and 47%. However, the order of prevalence would be different if the cutoff level set to 20 ng / mL. Using this threshold, the countries with the highest prevalence of vitamin D insufficiency is South Korea (64.4%), Lebanon (58.2%), Turkey (57.9%), Japan (47.0%), and Germany (33.0%). The lowest prevalence was found in Malaysia (11.3%), Thailand (12.0%), Sweden (12.7%), Brazil (15.2%), France (16.2%), and Chile (19.1 %).^{11,13,14}

Increasing public awareness about the negative effects of sunlight causes skin cancer has resulted in the fact that many people use sun protection and avoid sun exposure or conditions requiring to spend most of the time indoors. Lack of exposure to sunlight will cause the state of vitamin D deficiency Humans produce most of the vitamin D through exposure to the effects of UV rays on the skin. The use of a sunscreen with SPF > 8 will decrease the production of vitamin D by 95% [12].

Based on the test results of the three variables (25 (OH) D, estradiol and bone density) to a variety of risk factors, found meaningful results only on the levels of estradiol ($p = 0.001$) and bone density ($p = 0.016$) on the body mass index. These results prove that the body mass index influence on the formation of estradiol from the body ekstrasglandular, and the resulting increase in estradiol brought affect increases in bone mass, this is evidenced by the results of a significant positive correlation estradiol and bone density in this study.

A decrease in the activity of 25 (OH) D is a marker of vitamin D status is responsible for mass bone loss [13]. Given its role in maintaining the homeostasis of calcium and phosphate, as well as the effects of secondary hyperparathyroidism caused by vitamin D deficiency conditions that

would increase bone turnover, some studies try assess correlation of 25 (OH) D status bone density, the research Raya PM (2001) found significant correlation between bone density levels of 25 (OH) D serum ($r = 0.368$ and $p = 0.001$), but in this study there was no significant correlation between them with the value of $r = 0.261$ and $p < 0.05$, as well as the results obtained Tangpricha V (2004) with a value of $r = 0.01$ and $p = 0.53$. This difference may be due to the Kingdom PM et al study, the mean old subjects of the study had experienced menopause ± 13 years, based on the theory that there will be a decrease in bone mass by 2% since menopause occurs.

From the results of this study concluded that a significant positive correlation between serum levels of estradiol with a score of BMD, with the strength of the correlation $r = 0.53$ and $p < 0.05$, which means an increase in serum estradiol levels followed by elevated levels of bone density. This is in line with previous studies, some studies conducted in Indonesia is a Sulistio A (2013) research with $r = 0.456$ and $p = 0.001$ and Dewi N research with the value of $r = 0.39$ and $p = 0.001$. Based on existing theory, lack of the estrogen hormone is the etiology of primer to the occurrence of osteoporosis in postmenopausal women, in view of its function either directly or indirectly to the balance of formation and resorption of bone, with a decrease in average serum estradiol levels to the limit below 20pg / ml will affect the composition and bone density.

There is some increase in strength of the correlation in test double correlation between the levels of 25 (OH) D and serum estradiol to score bone density with a value that is meaningful, this supports that the levels of 25 (OH) D levels were positively correlated although the strength is weak and meaningless if the stand own.

V. CONCLUSION

The conclusion of this study is that there is no significant correlation between the levels of 25 (OH) D serum with a T-score, with the strength of the correlation $r = 0.261$ and $p > 0.05$.

There is a significant positive correlation between serum levels of estradiol with a T-score, with the strength of the correlation $r = 0.53$ and $p < 0.05$.

There is a significant positive correlation between serum levels of 25 (OH) D and estradiol together to bone density with the strength of the correlation $R = 0.663$ and $p < 0.05$. Influence of serum levels of 25 (OH) D and estradiol simultaneously on bone density score is 44%.

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