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Estimation of Some Mineral (Calcium, Phosphorous, Vitamin 25 (OH) D and Alkaline Phosphatase) in Osteoporosis Patients in Kirkuk City

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Abstract

Introduction: Osteoporosis is a growing public health problem of the elderly; it's qualified by low bone mineral density (BMD), with its associated increased risk of fractures. Despite the intensive researches, there are still large areas to explore with regard to its etiology.

Method and subject: We enrolled 100 female patients (40-80 years) in this study who attended the rheumatology Unit at Azadi teaching hospital/ Kirkuk city from February 2015 to July 2016 50 patents had osteoporosis confirmed by DEXA scan and the remaining ones who are age matched were controls. We measured Vitamin D, Calcium and Phosphorus and Alkaline phosphatase in the sera of the entire participant. A special questionnaire form was arranged and full information was collected from each subject include: name, age, sex, patients' weight and height were measured.

Results: One hundred women were enrolled in this study with mean age of (61.0 ± 10.2) years were studied; 50% of them were controls and 50% of them had osteoporosis.

Age of osteoporosis group were significantly higher than control groups (56 ± 9.0) years also BMI significantly higher ($p<0.001$) in osteoporosis (29.1 ± 5.9) compared to the control group (28.9 ± 3.4).

Both control and osteoporosis cases had calcium and phosphorus within normal range, however the difference between the levels was significantly higher in osteoporotic (8.89 ± 0.564) mg/dl than control groups (8.776 ± 0.496) mg/dl. Serum alkaline phosphatase was significantly higher in osteoporosis (96.89 ± 8.00) in comparison to the control group (81.0 ± 12.4) yet their values are still within normal range.

Interestingly the vitamin D deficiency was prevalent in the study population; it was significantly much lower in the osteoporosis group compared to the control group. According to chi-square test the age significantly associated with BMD, vitamin D, and phosphor in osteoporosis groups while in the control group the age was strongly associated with BMD only. The study showed correlation between Vitamin D and Bone mineral density.

Conclusion: We conclude that there was no significant association between serum Calcium, Phosphors, and alkaline phosphates with bone mineral density while show positive association between age and vitamin 25 (OH) D levels.

Keywords: Osteoporosis; Vitamin 25 (OH) D; Women; Alkaline phosphatase

Introduction

It is the most common metabolic bone disease. It is characterized by low Bone Mineral Density (BMD), loss of bone mass and micro architectural disruption of bone tissue that result in increased risk of fragility fractures, which are the main complications of the disease [1,2].

Osteoporotic fractures, especially of the vertebra, are common in the elderly and result in grief morbidity and mortality following minimal trauma [3].

Osteoporosis has become a major public health issue and it is rapidly increasing globally, especially in elderly Asians as the people are living longer [4,5].

The most common cause of osteoporosis is lack of physical stress on the bones because of reduced activity, others include malnutrition to the extent that sufficient protein matrix cannot be formed, lack of vitamin C which is necessary for secretion of intracellular substance by all cells. All the above impair formation of osteoid by the osteoblasts [6].

The three major osteoporotic fractures are those of the hip, vertebra and forearm, but fracture of the humorous, pelvis and rib are also common [7].

Vitamin D is a common medical condition worldwide. It is usually develops because of insufficient sources of endogenous and exogenous vitamin D (inadequate intake or excessive consumption) and may eventually result in bone mass reduction, especially in elderly woman [8].

Aging is associated with decreased; sun exposure, oral intake and skin activation of vitamin D, and vitamin absorption. All of these factors may contribute to vitamin D insufficiency, which is required for calcium absorption and bone mineralization. The low serum vitamin D is associated with increased PTH secretion which in turn leads to bone resorption and increased renal calcium excretion [9]. Vitamin D is essential for calcium metabolism as well as for fracture prevention and a recent review suggested that the optimal serum 25 (OH) D lies in the region of 50-80 $nmoL^{-1}$

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A strong association between vitamin D deficiency and fracture development has been suggested and thought to underlie the significant increase in mortality and morbidity rates of fracture patient [10,11].

Vitamin D status is typically determined by measuring serum 25-hydroxy vitamin D (25 (OH) D) level [12]. 25(0H) D is the best indicator of clinical status and is the key circulation vitamin D metabolite [13]. Pipe et al; found that in elderly patients who had recently experienced hip fracture, vitamin D insufficiency was a commonly detected phenomenon and not a coincidental finding [12].

Calcium is one of the major mineral components of the skeletal system and is also an essential nutrient for nerve conduction, muscle contraction, hormone and enzyme, secretion, and blood clotting. Adequate calcium intake is essential for normal growth and development of the skeleton and teeth and for adequate bone mineralization [14].

Calcium found primarily in bone (99%), blood and ECF. The overall goal of calcium haemostasis is to maintain biologically active ionized form with a narrow acceptable range (1.1-1.4 mmol/l) in order for critical function such as signal transduction to be performed [15]. Phosphate is considered to be a major dietary source of acid phosphate is supplied in generous amount in the diet through meat, grains products, and recently, it is added to food as additive [16]. While phosphate is the fundamental mineral component of hydroxyapatite, the principal structural element of bone the acid-ash hypothesis posits that dietary phosphate, a marker of metabolic production of acid is detrimental to bone [17-19]. So, additional variables may influence the relationship between dietary phosphate intake and bone health. Calcium intakes are, that is whether calcium intakes are limited or insufficient, might influence the relationship between phosphate, the diet acid load, and bone health [20,21]. Alkaline phosphates has been clinically available for several years as a marker for hone metabolism serum alkaline phosphatase consists of several dimeric isoforms that originate from various tissues such as liver, bone, intestine, spleen, kidney, and placenta. In adult with normal liver function. Approximately 50% of the total Alkaline Phosphatase (AlP) activity arises from liver and 50% from the bone [22].

Aim of the Study

The aim of present study is to evaluate Vitamin D and to show it is effect on bone loss and measure Ca ion and it is role in bone loss. Finally we will measure phosphate and alkaline phosphatase and their effect on the bone loss.

Materials and Methods

This cross-sectional study was carried out on 100 subjects (42-80 years) at the Rheumatology Unit at Azadi Teaching Hospital, Kirkuk city from February 2015 to July 2016. We included, women attending outpatient clinic at Azadi Teaching hospital for screening for osteoporosis by DEXA scan following clinical examination by specialist doctors. A special questionnaire form was constructed and full information was collected from each subject including: name, age, sex, patients' weight and height. A special attention was paid if there was a previous history of hip or vertebral facture, hip replacement, smoking, Glucocorticoids intake, rheumatoid arthritis, alcohol drinking, drinking milk, coffee, tea, if they do exercise or not and excluded women from the study if they had any disease like rheumatoid arthritis or chronic obstructive pulmonary disease or received any anti-osteoporosis treatment or they had disease affected on ALP. 5 mm of venous blood were collected in plane tubes and centrifuged at 3000 rpm for 5 min and the serum were kept in deep freezer (-20°C) until analysis.

Total Calcium and Phosphorus were measured in serum samples using spectrophotometer based method using kits provided by biomeurx (France) according to the supplier instructions. AIP was measured using Reflotron plus machine (Roche) with its costume Kit. Vitamin D nutritional status is best determined by the measurement of 25 (OH) D rather than 1,25 (OH) D because 25 (OH) D is main circulating form of vitamin D and day variation is less due to its long half-life and it is measurement easier than the other ones [23]. Vitamin D estimate was carried out in private laboratory using minividas machine from (France) using it's provided kit specific for 25 hydroxy cholicalciferol levels were classified as sufficient (≥ 30 ng/ml), insufficient (11-29 ng/ml) and deficient (≤ 10 ng/ml) [24] . It's measurement of bone mineral density was performed by Duel Energy X-ray Absorptionmetry (DEXA-Scan) the results were reported according to the WHO classification of BMD T-score. BMD was measured at femoral neck on the right and left side and the lumbar spines (1 L-4 L). It classifies the patient as normal between 1.0 and -1.0, osteopenia -1.0 to -2.5, osteoporosis -2.5 or lowers this value [25].

Statistical Analysis

Statistical software SPSS version 25 (Chicago, IL) was used for data input and analysis. Two sided student t-test was used to compare the mean value of the biochemical tests in different groups. The Pearson correlation coefficient analysis was used to show the correlation with age, biochemical parameters and BMD. For all statistical analysis p-value less than 0.05 were considered statistically significant.

Results

One hundred women were enrolled in this study with mean age of 61.0 ± 10.2 years; of them 50 were controls and the rest of the study population were confirmed to have osteoporosis.

Table 1 show Mean and Standard Deviation (SD) of Age, BMI, serum alkaline phosphatase, calcium and phosphorus, Vitamin D2 (OH) and DEXA ray in osteoporosis patients and controls group. BMI of osteoporosis group (28.9 ± 3.4) was significantly ($p<0.001$) lower than control groups (29.1 ± 5.9). The calcium levels were significantly higher in control (8.89 ± 0.564 mg/dl) than osteoporotic group (8.776 ± 0.496 mg/dl) and phosphorus levels in control groups were (3.34 ± 0.53) significantly lower than osteoporotic group (3.78 ± 0.4).Both control and osteoporosis women had serum calcium and phosphorus level within normal range. Serum Alkaline phosphatase was significantly higher in osteoporosis (96.89 ± 8.00) than control groups (81.0 ± 12.4) yet it is still within normal range. Interestingly vitamin D deficiency was common in both control and osteoporosis, it was significantly much lower in the later group compared to the control group.

Parameters	Osteoporosis N=50	Controls N=50	P-Value
	Mean ± SD	Mean ± SD	
Age/ year	61.0 ± 10.2	56 ± 9.0	0.000
BMI kg/m²	29.1 ± 5.9	28.9 ± 3.4	0.000
Alkaline phosphatase mg/dl	96.89 ± 8.00	81.0 ± 12.4	0.000
Calcium mg/dl	8.89 ± 0.564	8.776 ± 0.496	0.26
Phosphor mg/dl	3.78 ± 0.4	3.34 ± 0.53	0.000
Vitamin 25 (OH) D ng/ml	13.25 ± 5.12	19.99 ± 8.88	0.000
DEXA g/cm²	-3.27 ± 0.76	-0.97 ± 1.01	0.000

T-test P=<0.05 Vitamin 25 (OH) D=25 (hydroxycholecalciferol) N=number

Table 1: Mean and standard deviation of age, body mass index (BMI), serum alkaline phosphatase, calcium, phosphorus, vitamin D2 (OH), and DEXA ray in osteoporosis patients and controls group.

According to BMD classification, the number of women who had osteoporosis was 50 with a T score of 3.278 ± 0.757, twenty three of women were osteopenia (T score 1.9 ± 0.407) and twenty seven of them was normal BMD with Mean ± SD (0.321 ± 0.492).

Table 2 shows the level of serum vitamin 25 (OH) D in study group women who appear that there were (34) of them below ≤ 10 ng/ml had deficient the Mean ± SD (8.712 ± 0.756) while the number of women were ranging between 11-29 ng/ml was (59) Mean ± SD (4.455 ± 5.269) had insufficient and the last ones had sufficient their number was (7) and Mean ± SD was (33.114 ± 6.201).

According to chi-square test (Tables 3 and 4) shows the age significantly difference with BMD, vitamin D, and phosphorus in osteoporosis groups while in control groups the age significantly with BMD, respectively.

According to Pearson correlation there was inverse relation between bone mineral density and alkaline phosphates and serum phosphorous; but there was positive relation with vitamin 25 (OH) D $P<0.05$, while there was negative relation with calcium ion $P>0.05$ (Table 5).

Serum vitamin 25 (OH)D ng/ml		
≥ 30 ng/ml Sufficient	**11-29 ng/ml Insufficient**	**≤ 10 ng/ml deficient**
Mean ± SD N=7	**Mean ± SD N=59**	**Mean ± SD N=34**
33.114 ± 6.201	4.455 ± 5.269	8.712 ± 0.756

N=Number, 25 hydroxycolicalcifirol vitamin D3

Table 2: Classification of serum vitamin 25 (OH) D levels in study groups (N=100).

Age / Parameters	χ^2 computed N=50	χ^2 tabulated N=50	DF	P-Value
BMD	24.96	21.03	12	S
Vitamin D	25.00	24.99	15	S
P	37.91	21.03	12	S
Ca	22.48	26.29	16	NS
AIP	11.22	21.03	12	NS

χ^2=Chi test, DF=Degree freedom, P-value=P<0.05

S: Significant; NS: Non-Significant; N: Number

Table 3: Shows the relation between age and parameters in osteoporosis patients.

Age / Parameters	χ^2 computed N=50	χ^2 tabulated N=50	DF	P-Value
BMD	38.31	26.29	16	S
Vitamin D	32.15	36.42	24	NS
P	12.82	21.03	12	NS
Ca	15.497	21.03	12	NS
AIP	28.45	31.41	20	NS

χ^2=Chi test, P-value=P<0.05

DF: Degree Freedom; S: Significant; NS: Non significant; N: Number

Table 4: Shows the relation between age and parameters in control groups.

Biochemical marker	BMD Pearson correlation	*P*-value
Alkaline phosphates (mg/dl)	-0.724	0.000
Calcium (mg/dl)	-0.186	0.065
Phosphorus (mg/dl)	-0.270	0.007
Vitamin 25(OH)D (ng/ml)	0.639	0.000
Age years	-0.560	0.000

Significant=P<0.05

Table 5: Pearson correlation between BMD and biochemical markers for the study group (100 women).

Discussion

It is fact the world population is getting older, this issue brought osteoporosis to the attention of the scientist as it is known to be the disease of elderly [1,2]. Many studies tried to identify the etiology of osteoporosis or at least recognize its risk factor [1,2].

We showed in this study the negative effect of increasing age on the BMD, this agree with study done by Faiq [26] who conducted a study in Baghdad and found that older age women had significant lower BMD. Another study done by Sasmita et al. [27] showed that prevalence of osteoporosis increase with age.

The data of this study showed no significant difference in the level of serum Calcium between osteoporosis group (8.89 ± 0.569 mg/dl) and control group (8.77 ± 0.496 mg/dl) similarly, there was no relation between BMD and calcium ion. Our work agrees with Rana [28] who found no significance difference in calcium level in osteoporotic women (2.19 ± 0.11 mmol/l) and in control group (2.24 ± 0.14 mmol/l) the normal range in mmol/l (2.1-2.6).

In the current study serum phosphorus showed significantly difference between osteoporosis groups (3.78 ± 0.4 mg/dl) and control groups (3.34 ± 0.53 mg/dl) but their value still within normal range for this is agree with Selvapandian et al. [1] and also showed no significant difference in the level of serum phosphorus for study women groups (6.39 ± 1.14 mg/dl) also study done by Rana [28] found that osteoporotic group (1.13 ± 0.19 mmol/l) and control (1.15 ± 0.17 mmol/l) the normal range in mmol/l (0.8-1.6), this may be serum Ca and P was regulated and homeostasis is maintained in serum regardless of their store in bone relation of phosphorus with BMD [29].

Serum alkaline phosphatase significantly difference between osteoporotic groups (96.89+8.00 mg/dl) compared to control group (81.0 ± 12.4 mg/dl) but their value within normal range that mean there was no significant relation between osteoporosis and alkaline phosphatase also agree with study done by Rana [28] (63.43 ± 12.91U/l), (64.28 ± 13.5 U/l) and Selvapandian et al. [1] India for all women (128.7 ± 29.77 U/l) while disagree with Ramesh et al. [30] who showed raised level in alkaline phosphatase. This may be due to AIP can be drain from osteoblast which is rich with AIP also it found in plasma membrane of the cell in the liver, intestine, and placenta, all of which is may contribute to the total amount of alkaline phosphatase. While evaluating the vitamin D status of the study 59% of women had vitamin D insufficient which agree with Wei et al. [31] 64.7% of women had vitamin D insufficient; in our study 34% of the women had deficiency this agree with study done by Francisco et al. [32] 24% of their study groups below 25; Ramesh et al. [30]. Found that 25 (OH) D levels revealed 62% of their patient had vitamin D deficiency and four subject had strongly vitamin D deficiency, in study done in Saudi by Alkhenizan et al. [33] that found 36.4% had insufficiency moderate to 8.6% severe vitamin D deficiency and, in study done by Pedro et al. [34]; that found no significant difference between vitamin 25 (OH) D, age group and BMD. The decrease of serum vitamin D levels may be due to less outdoor activities of the women also with decrease exposure to sun light and due to our habits in wearing long dress this will prevent the vitamin D in the skin [35] to induce and convert to the active form which is the major storage form of vitamin D consecutively it is important for calcium and phosphorus absorption, when it is level decrease lead to increase parathyroid hormone which lead to decrease calcium absorption then effect on bone health.

Conclusion

We concluded from this study the BMD significantly related with age and BMI which the elderly women with low BMI were had more risk factor to osteoporosis and fracture than those younger and normal BMI; in addition serum calcium, phosphorus and alkaline phosphates were not significantly be affected by BMD, in this study osteoporosis correlated with vitamin 25 (OH) D which is the major factor and the active form of vitamin D. Vitamin D deficiency is common in our population (93%) of osteoporotic and control group have low vitamin D.

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