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Original Article

Effect of Some Trace Minerals on Serum Osteocalcin Level in Iraqi Osteoporotic Postmenopausal Women

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ABSTRACT

Objectives: There has been no study to detect the relation of some trace minerals with osteocalcin levels in progress of osteoporosis in the postmenopausal women. The aim of this study is to evaluate whether zinc and copper levels associated with osteocalcin levels in Iraqi postmenopausal women had osteoporosis

Methods: A study of case with control included 85 postmenopausal women. Their ages ranged from 45-70 years, based on the T- score for BMD, and these subjects were divided into two groups: patients with osteoporosis(n=45) and controls without osteoporosis(n=40).

Results: A significant difference between osteocalcin levels in osteoporotic and non-osteoporotic postmenopausal women ($p < 0.001$) was observed, as well as a significant difference of zinc levels was observed ($p<0.001$) between the two previous groups. The values for age ($P = 0.003$),weight ($P = 0.041$) and height ($p = 0.005$) were statistically different in comparing patients group to controls group, while the difference of BMI values ($p = 0.409$) was not significant between the two previous groups. There was no significant difference for serum levels of 25-OH vitamin D, copper, calcium, phosphorous, and protein, between patients and controls. The correlation of osteocalcin with zinc ($r = - 0.474$), menopausal duration($r = 0.288$) and age ($r = 0.217$) was significant.

Conclusions: A significant increase of osteocalcin levels and significant decrease in zinc levels revealed an apparent increase in the bone turnover of osteoporotic postmenopausal women.

Keywords: Osteocalcin, osteoporosis, postmenopausal, zinc, copper, 25-OH vitamin D

1. INTRODUCTION

Osteoporosis is a disorder affecting entire skeleton that results from bone remodeling process imbalance causes decreasing in strength of the bone and hence susceptibility of fracture increased[1]. Osteoporosis appears silently without symptoms, the patient does not realize having an osteoporosis until a fracture is taken place [2]. It is rated that over the age of 50 years about one of three women and one

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of five men will undergo from a fracture of osteoporosis[3].Throughout life, the bone remodeling and modeling is adjusted the structure and the material composition of bone to controls loads.

These two paths constitute a structure able to conserve its strength during growth. While factors accompanied advancing age, like hormonal imbalance, imbalance of growth factors, reduced the mobility and muscle mass, an inappropriate nutrient and some other factors that affect capability of the remodeling process to maintain bone strength and controls bone loads[4]. Whenever we aging, the bone decays in structure, function, and texture which lead to reducing bone tissue mass and causing osteoporosis. Imbalance between formation of new bone and resorption of the bone accelerates the bone mass loss. In menopausal women there is noticeable increase in resorption of bone more than formation of the bone, resulting from declining estrogens levels to significant low amount. Thus inducing and accelerating the bone mass loss because of turnover from the osteoblastogenesis to prevalent adipogenesis in bone marrow. This has a lipotoxic effect that negatively affects the bone mineralization and matrix formation[5].

The bone loss in women happen in two stages: The first appears prevalently in trabecular bone and beginning at menopause resulted from estrogen deficiency, and leads to increase in the resorption of bone in comparison with bone formation. This stage could be specified as menopause related to bone loss. The second stage appears after4-8 years and demonstrates a continual, slower loss of both cortical and trabecular bone, and is mainly due to decreased bone formation, also defined as age associated bone loss, which is the only stage that occurs in men [6].

2. METHODS

This study was conducted in Kirkuk city, Iraq from November 2017 to March 2018. The subjects were selected from women at menopause for more than one year who voluntarily had visited the rheumatology and physiotherapy clinic at Azadi teaching hospital. Approvals were given from the research committee of health directorate of Iraqi health ministry to conduct the study according to ethics committee for health foundations.

2.1. Characteristics of participant women

This study involved a total of 85 postmenopausal women, their ages range from (45-70) years, the randomly selected women divided in to two groups: patients [postmenopausal women had osteoporosis (N = 45)] were diagnosed according to the World Health Organization (WHO) diagnostic guidelines of osteoporosis status; a T.score of BMD ≤ -2.5 SD is considered osteoporosis by measuring bone mineral density (BMD) via central DXA (3D) scan (dual energy x-ray absorptiometry) from Stratos Dr (DMS, France) at lumbar spine L1-L4, and hip, and the second group was controls [postmenopausal women without osteoporosis (N= 40)], according to WHO if the T. score of BMD ≥ -1.0 SD is regarded normal. Participants were excluded from the study with the following criteria: Diabetes mellitus, hyperthyroidism and hypothyroidism, epilepsy, prolonged use of drugs that affect bone metabolism, liver or renal diseases, malignancies, inherited disorder or rheumatoid arthritis, smoking. Each participant gave their approval for included in this study.

2.2. Anthropometric data

An interview with participants was done and information related to this study was given from each participant including the age, weight, height, menopausal duration, medical history. The height of all subjects was measured in (cm) by standardized customary methods without shoes from top of the head to the heel of the feet and the weight was weighed in (kg) with light clothes by weighing scale (mechanical bathroom from Salter doctor). The Body Mass Index (BMI) has been calculated by division Weight (kg)/Height (meter) ^{2} for each participant.

2.3. Sample collection and analysis

All the matched subjects for this study were identified by numerical codes. 5 ml of whole blood was collected by venipuncture from each subject after overnight fasting, and drawn into free anticoagulant tube to allow clotting at room temperature. Within 1 hour of started blood collection process, the sample was centrifuged at 3000 rpm (round per minutes) for 10 minutes to separate serum from other blood cells components. The collected serum was kept into aliquots. The serum was immediately stored and frozen at -30^{0}C until tested. The serum samples were clear, non-hemolytic, non-lipemic and non-icteric can be used; frozen serum can be thawed only once for analyzing serum osteocalcin by ELISA kit for human from the Bio Vendor Medicine Laboratory. Inc., Brno, Czech Republic, the total vitamin D by ELISA kit for human purchased from LDN, Germany , while zinc and copper by colorimetric test from Centronic GmbH/ Germany. The serum zinc reacts with 2-(5-Brom-2-pyridylazo)-5-(N-propyl-N-sulfo-propylamino)-phenol and forms a red chelate complex. This method does not need deproteinization of the sample. The measurement absorbance is proportional to total zinc concentration in the sample [7].At pH of 4.7,the copper is released from the carrier protein, and react with 4-(3, 5-Dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylaniline to form a chelate complex. The absorbance of formed complex is equivalent to a total copper concentration in the sample[8].

2.4. Statistical analysis of data

All data were statically analyzed by using SPSS software version (24, Inc., Chicago IL, USA). The descriptive statistics were performed as mean ± Standard deviation SD, probability and p-value test for significant variation between the patient group and control group was analyzed by using the T-test. The results were analyzed statistically for normal distribution (by Kolmogorov-Smirnov and Shapiro-Wilk tests), outliers, random number (both were detected by P-P

plot and Q-Q plot), and homogeneity of variance (by levene statistic test), before applied the T-test, one way ANOVA, and Pearson correlation. AP-value <0.05 or P- value <0.01were considered statically significant at 2- tailed.

Table 1: Anthropometric and biochemical parameters of the study population

Anthropometric Parameters	Patient (n=45) Mean±SD	Control (n=40) Mean±SD	P-value
Age (year)	61.44±5.79	57.90±4.67	0.003
Weight (kg)	70.82±11.61	75.97±11.16	0.041
Height (cm)	154.60±6.57	158.22±4.84	0.005
BMI (kg/m^2)	29.54±4.65	30.35±4.29	0.409
Menopausal duration (yrs.)	11.66±4.93	7.42±4.15	<0.001
Biochemical parameters			
Osteocalcin (ng/ml)	20.10±5.33	12.01±2.36	<0.001**
25-OH vitamin-D (ng/ml)	20.94±8.46	24.94±11.13	0.064
Zinc (µmol/l)	9.42±2.27	12.27±2.08	<0.001**
Copper (µmol/l)	18.24± 5.64	15.97± 5.87	0.073
Calcium (mmol/l)	2.10± 0.29	2.15± 0.28	0.433
Phosphorus (mmol/l)	0.93± 0.21	0.99± 0.17	0.115
Protein (g/l)	69.13± 6.98	69.60± 5.55	0.737

* P< 0.05 is significant at the level 0.05
** P < 0.01 is significant at the level 0.01

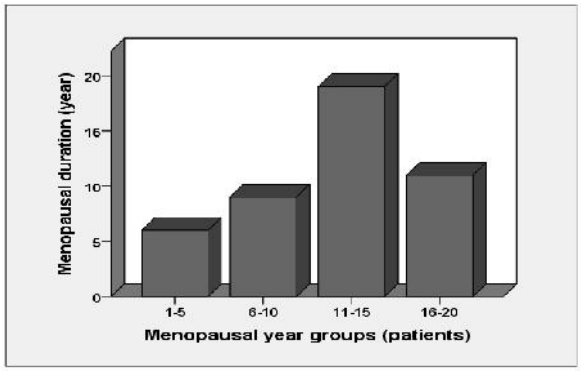

Fig 1: Distribution of menopausal years in patient group according to age groups.

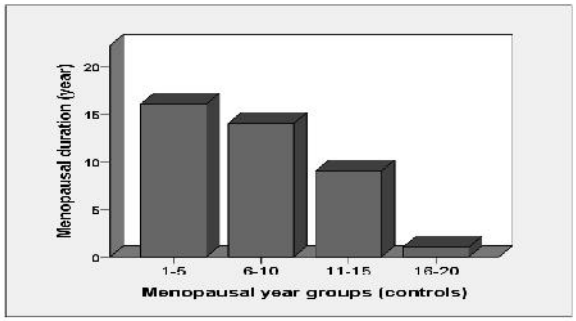

Fig 2: Distribution of menopausal years in control group according to age groups.

Table 2: Prevalence of study population according to age groups

Age (years)	45-50 y	51-55 y	56-60 y	61-65 y	66-70 y
Patient N (%)	3 (3.5)	7 (8.2)	7(8.2)	16 (18.8)	12 (14.1)
Control N (%)	3 (3.5)	13 (15.3)	11 (12.9)	10 (11.8)	3 (3.5)

Table 3: Comparisons of biochemical parameters within the same age groups between patients and controls

Biochemical parameter	Groups	45-50 y Mean ± SD	51-55 y Mean ± SD	56-60 y Mean ± SD	61-65 y Mean ± SD	65-70 y Mean ± SD
Osteocalcin ng/ml	Patient	21.96±4.01	22.79±2.75	18.20±2.20	19.07±3.45	20.53±2.99
	Control	11.54±4.37	11.72±3.35	12.52±2.44	12.04±3.04	11.77±2.40
	p-value	**0 .039***	**<0.001***	**0.001***	**<0.001***	**0.002***
25-OH vitamin D (ng/ml)	Patient	20.82±13.74	24.31±5.23	17.86±7.31	21.51±8.60	20.03±9.60
	Control	24.21±12.73	22.28±13.67	23.61±10.41	28.07±8.48	31.64±9.812
	p-value	**0.494**				
Zinc (µmol/l)	Patient	8.34±3.74	9.46±3.32	9.60±1.44	9.64± 2.01	9.27±2.22
	Control	10.45±1.17	11.19±1.34	13.54±1.83	12.51±2.35	13.31±2.65
	p-value	**0.405**	**0.029***	**<0.0.1***	**0. 003***	**0. 017***
Copper (µmol/l)	Patient	13.74±6.21	18.23±4.80	17.28±5.62	17.19±5.06	21.31±6.14
	Control	15.12±7.24	15.08±5.41	15.71±6.42	18.44±5.92	13.41±5.70
	p-value	**0.222**				

* P< 0.05 significant at level the level 0.05

Table 4: Pearson correlation between osteocalcin levels and other variables

Parameters	Pearson correlation (r)	P-Value
Menopausal duration (yrs.)	0.288	0.007**
BMI	-0.156	0.155
Age (yrs.)	0.217	0.046*
25-OH vitamin-D (ng/ml)	-0.191	0.079
Zinc(µmol/l)	-0.474	<0.001**
Copper (µmol/l)	0.184	0.093
Calcium (mmol/l)	-0.058	0.598
Phosphorus (mmol/l)	-0.024	0.830

* P< 0.05 or ** P < 0.01 significant at the level 0.05 or level 0.01 respectively
BMI(body mass index)

3. RESULTS

3.1. *Anthropometric characteristics of study population*

The major characteristics of 85 postmenopausal women who were matching the inclusion criteria are shown in Table 1. It has been founded that mean age of the patient group was (61.44±5.79) year, while the mean age for the control group was (57.90±4.67) year. There was significant difference in the age, height, and weight but not BMI. To indicate the difference of the years of menopausal duration in patient and control groups, the menopausal years for both patient and control groups, were classified into four groups: first (1-5) years, second group (6-10) years, third group (11-15) years and fourth group (16-20) years. The high frequency of the menopausal duration for patient group was between (11-15) years, while for control group the high frequency of menopausal duration was between (1-5) years, and (6-10) years subsequently (Figure 1 and Figure 2). The concentration of serum osteocalcin of age (45-50) year for the patients and controls was significantly different, while there was no significant difference in serum Zinc levels of the same age(45-50) group for patients and controls. It was found that both osteocalcin and zinc concentration was significantly different in age groups (51-55), (56-60), (61-65), (66-70) for patients and controls. The difference in

serum copper, 25-OH vitamin D, concentrations among all age groups for both patients and controls was not significant (Table 2 and 3). The correlation of serum osteocalcin levels with menopausal duration and age was positive and significant (p= 0.007), (p= 0.046) respectively. On other hand the correlation of serum OC levels with serum zinc levels was inverse, and significant (p=<0.001), but the correlation of serum OC levels with serum copper, serum 25-OH vitamin D, serum calcium, serum phosphorous, serum protein levels was and not significant.

4. DISCUSSION

This study evaluates whether some trace minerals associated with osteocalcin levels in Iraqi postmenopausal women had osteoporosis when compared with postmenopausal women not having osteoporosis. As we see in the present study, the patients were significantly older and consent with a study conducted in Iran which identified age as a significant risk factor for osteoporosis in women[9]. Also agreement with another study done in Morocco and reported that the age was the main predictor of BMD at the total hip[10].

The study revealed that height and weight of controls were slightly more than patients and were statistically significant; it seems likely that weight gain(no one of the subjects, their weight exceeds 100 Kg) will stand up against bone loss in postmenopausal women and this consent with the Framingham study of osteoporosis that reported if women during the period of interim were gained weight, they gained bone mineral density(BMD)or had little change in the BMD [11]. Also consent with many previous studies [12, 10].

Although the significant difference in the height and weight as described above, the BMI of controls was slightly more in patients and the difference was not significant. Our data indicate the presence of osteoporosis among individuals with low BMI; and agreement with previous studies[13, 14].

The menopausal duration for patients was significantly elevated than controls. The present study showed that the high frequency of the menopausal duration for patient group was within (11-15) years while for control group was (5-10), and this revealed that the risk of osteoporosis developing will increase whenever the duration of menopause will lengthen due to long estrogen deficiency, and is in accordance with previous studies [15, 16]. If the period of postmenopausal is more than 5 years it will show the risk factors of osteoporosis[17].

The present study showed that serum osteocalcin level (OC) was significantly higher in patients than controls, and this accordance with many previous studies[18, 19]. Serum OC level revealing an inverse relationship with BMD, and considers a promising marker for bone turnover, which showed to be increased in postmenopausal women, and may be used in diagnosis of primary osteoporosis[20, 21]. Furthermore the serum OC concentration was significantly higher in patients than controls in all age groups (45-50, 51-60, 61-65, 66-70), this demonstrates effective evidence that serum OC level can predict BMD in postmenopausal women. After the fifth decade, the osteocalcin level in postmenopausal women increasing with age, resulting from increased bone turnover rates and subsequently leading to higher osteocalcin synthesis[22]. Therefore osteocalcin can be used as a sensitive marker to indicate and to evaluate the bone metabolism as well as the BMD in postmenopausal women [23].

The serum concentration of 25-OH vitamin D was higher in controls than osteoporotic postmenopausal women but not significantly differs and this accordance with many previous studies [24, 25]. Also the concentrations of 25-OH vitamin-D were not significantly different within the age groups that mentioned above in both osteoporotic and non-osteoporotic women postmenopausal women. Although the difference was no significant but our data demonstrated that 25-OH vitamin D(vitamin-D) deficiency was common among osteoporotic postmenopausal women. 8.9% of osteoporotic postmenopausal women have vitamin-D deficiency and 73% have insufficiency levels of vitamin-D, while the non-osteoporotic postmenopausal women; 10% have deficiency levels of vitamin-Dand 27.5% have vitamin-D insufficiency. Even the mean values of 25-OH vitamin D for osteoporotic and non- osteoporotic postmenopausal women were within insufficiency status. Regarding the normal 25-OH vitamin D status is defined as the concentration> 30 ng/ml, while 25-OH vitamin D deficiency is defined as concentration < 10 ng/ml, and insufficiency25-OH vitamin D status is recognized as concentration <30 ng/ml [26]. The present study showed the serum zinc (Zn) concentration was significantly lower in osteoporotic postmenopausal women than controls, provided convenient evidence that the diagnosed Iraqi postmenopausal women with osteoporosis had low serum zinc. In a study done in culture cells, revealed that zinc increases the cellular proliferation of osteoblastic cells (MC3T3- E1) during 1-10 days, as a time which is considered osteoblasts stimulating differentiation of synthesis of marker bone protein [27]. Also the study data revealed that serum zinc concentrations were significantly greater in osteoporotic postmenopausal women than controls according to these age groups (51-60, 61-65, 66-70), but in age group(45-50) the difference was not significant. These data demonstrated that zinc levels began to decline after age 50 year. Interpreting these changes may be because of urinary zinc excretion. Elderly women excretes more zinc in urine than young women, and the degree of increasing urinary zinc excretion in osteoporotic postmenopausal women relates to osteoporosis severity and therefore zinc may be used as a marker of bone metabolism changes [28].

Increasing copper (Cu) can have negative impacts, and it produces free radicals, which lead to induce lipid peroxidation, and result in intruding with bone metabolism [29]. The elevated levels of serum copper in patients than controls were not significantly differ. Also the serum copper levels were not significantly differing between the age groups in both patient and control groups. Increasing copper

in patients reveals the oxidative properties of Cu in patients may play role in development of osteoporosis, while zinc acts as anti-oxidative and prevent osteoporosis progress. Decreasing Zn levels and increasing Cu levels in this study reveal an unbalanced zinc and copper, which commonly take place in diseases depending on age, and it is thought to result in lipids oxidation, which in turn constitutes the integrity of neuronal and vascular membranes. In gathering with other factors, oxidative stress-mediated via trace metal imbalances could participate to the increase in vulnerability to the development of degenerative diseases with age [30]. This finding accordance with previous study showed more copper levels in osteoporotic women than non- osteoporotic women and it also was not significant [31].

The study data revealed that both calcium and phosphorus levels of controls was slightly higher than patients with no significant difference. Similar study have been reported that no significant correlation between the levels of serum calcium and phosphorus with osteoporosis [32]. Our data revealed that approximately 18% and 15.7% of patients had slightly low levels of calcium and phosphorus concentrations respectively; this may be after menopause and because of estrogen deficiency, result in calcium loss by indirect impacts on extra skeletal calcium homeostasis as well as reduced calcium conservation by kidney and decrease intestinal absorption of calcium[33, 34].

The present study did not demonstrate a significant difference in protein levels between patients and controls. On other hand this study revealed that 15.4% of patients had serum protein levels below the normal range if considering the cutoff minimum normal range is (60g/l), and these levels ranged from (54.50−59.80)g/l. While in controls, none of the women had serum protein level lower than the minimum normal range. Decreased serum protein levels in osteoporosis postmenopausal women result from aging, or the cause might be insufficient intake of proteins which lowered protein levels. These causes may take part in the occurrence of osteoporosis. Similar statement was reported by previous studies [35, 36].

The present study showed that the osteocalcin levels were correlated positively with age and consent with many previous studies [35, 37].Serum osteocalcin levels increase with age, and women above 65 years old have almost 2 fold higher osteocalcin levels when compared to women less than 44 years old [38]. The duration of menopause correlated significantly with serum levels of osteocalcin [Table4 /Fig 3] the same statement was made by another previous study conducted in India [15].

To our knowledge, at less in Mediterranean region, this is the first study identified an inverse association of serum osteocalcin levels with serum zinc levels. The serum zinc levels showed a highly significant negative correlation with serum osteocalcin levels. This data demonstrated that decreased serum zinc levels leads to increased serum osteocalcin levels in patients. It is suggested that low zinc can decrease osteogenic cells effect and this decreases cell proliferation, inhibition of ALP and collagen synthesis; this is compensated by an increase in osteocalcin level [27].

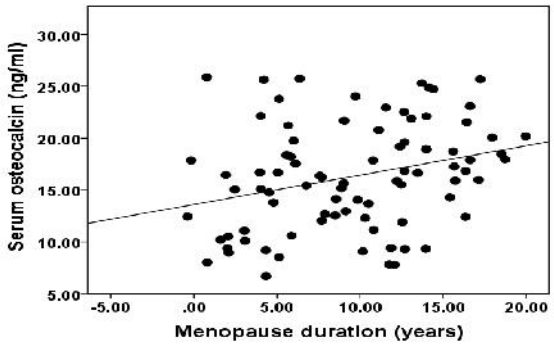

Fig 3: Scatter plot demonstrating the correlation between osteocalcin levels and menopausal duration

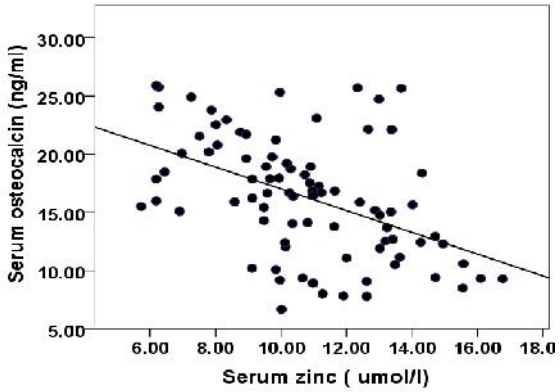

Fig 4: Scatter plot demonstrating the correlation between osteocalcin levels and zinc levels

5. CONCLUSIONS

A significant increase in serum of osteocalcin levels and significant decrease in serum of zinc levels revealed an apparent increase in bone turnover in osteoporotic postmenopausal women compared to postmenopausal women who had no osteoporosis. This demonstrated that serum osteocalcin level could be used as a biochemical marker and serum zinc level as a biochemical parameter for the extra diagnosis of osteoporosis in the Iraqi postmenopausal women after diagnosis and scanning with DXA. Further studies are needed for measurement of other bone mineral contents, and to detect and understand the role of trace elements in slowing the rate of loss of bone in postmenopausal women.

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