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Evaluation of Serum Osteocalcin and Osteopontin Levels as Bone Biochemical Markers in Postmenopausal Women

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Background: Studies discussing the correlation between biochemical markers of bone turnover and the diagnosis of osteoporosis in postmenopausal Iraqi women are rare. This study is devoted to find out the significance of serum osteocalcin (OC) and osteopontin (OPN) levels with the incidence of osteoporosis and its major complication (fracture).
Objectives: to investigate the significance of bone turnover biochemical markers; serum osteocalcin and serum osteopontin in evaluating osteoporosis for postmenopausal Iraqi women with and without history of vertebral fracture, as well as to explore the relationship of these markers with bone mineral density (BMD).
Methods: Fifty seven postmenopausal women whose ages are fifty years old and over, categorized into two groups: osteoporosis postmenopausal women (n=30), and healthy postmenopausal women (n=27). All sera samples were analyzed for serum alkaline phosphatase, calcium and phosphorous by using spectrophotometric kit. Serum OC and OPN levels were measured by immunoassay (ELISA) kits. BMD were measured by (DEXA).
Results: BMD and T-score were significantly lower in osteoporotic postmenopausal group as compared with healthy group (p=0.0001). Serum OC levels and serum OPN levels were elevated significantly in osteoporotic postmenopausal group as compared with healthy group (p=0.0001).
Conclusions: The levels of OC and OPN in serum could be used as a biochemical indicator in the early diagnosis of osteoporosis postmenopausal women.

Introduction:

Osteoporosis is a progressive systemic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture [(1)]. Osteoporosis itself has no symptoms; its main consequence is the increased risk of bone fractures (particularly vertebral compression fractures and hip fractures). The compression fractures in the spine that are caused by weakened vertebrae can lead to pain in the mid back. These fractures often stabilize by themselves and the pain eventually goes away. But the pain may persist if the crushed bone continues to move around and break.

A lack of estrogen in postmenopausal women prevents the absorption and utilization of calcium and is the single most important factor in the development of osteoporosis in elderly women [(2)].Menopause and ageing is associated with accelerated loss of cortical bone. Bone loss occurs when the balance between formation and resorption is upset [(3)].

Bone pain may result from a pathological reason such as certain metabolic bone disease or malignancy, in the absence of obvious fracture. The diagnosis of osteoporosis can be made using conventional radiography and by measuring the bone mineral density (BMD). In addition to the detection of abnormal BMD, the diagnosis of osteoporosis requires blood tests investigations.

Biochemical markers of bone turnover measured in plasma or urine are proteins or products derived from them. In general they are either enzymes derived from osteoblasts involved in bone formation, or from osteoclasts involved in bone resorption, or are constituents of the bone matrix, which escape into the circulation during the process of bone formation, or which are released as breakdown products during resorption. Bone turnover is caused by two functions; production of new bone, and the loss of old bone. The amount of bone mass depends on the balance between these functions, which is the bone turnover rate. If bone production is less than the amount of bone being resorbed, the risk of developing osteoporosis increases. Biochemical markers of bone turnover reflect the status of bone metabolism in various processes coupled with bone resorption and formation, and are widely used in clinical situations to evaluate the efficacy of treatments for osteoporosis [(4)].

Several population-based epidemiological studies have shown that bone turnover markers can predict bone loss and the incidence of osteoporotic fractures in women [(5)]. Individuals with increased bone turnover markers lose

bone at a faster rate than subjects with normal or low bone turnover markers [6], because of the physiological characteristics; the morbidity of osteoporosis is obviously higher in postmenopausal females than in males [1].

Osteocalcin (OC) is a small protein, 49 amino acids long (5.8 kDa). It includes 3 residues of gamma-carboxyglutamic acid. It has a negatively charged surface that places five calcium ions in positions complementary to those in hydroxyapatite (HA), an important mineral component of bone. OC is the most abundant non-collagenous protein in bone, comprising almost 2% of total protein in the human body. It is important in bone metabolism and is used as a clinical marker for bone turnover. The strength of bones mainly comes from the hexagonal mineral hydroxyapatite (HA) [7].

Osteocalcin is secreted exclusively by osteoblasts to play a role in the body's metabolic regulation and is bone-building, by nature. It is also involved in bone mineralization and calcium ion homeostasis [8]. As OC is produced by osteoblasts, so it is often used as a marker for the bone formation process. It has been observed that higher serum OC levels are relatively well correlated with increases in BMD during treatment with anabolic bone formation drugs for osteoporosis.

Osteopontin (OPN) is a highly phosphorylated sialoprotein that is a prominent component of the mineralized extracellular structural protein and an organic component of bones and teeth [9]. Osteopontin is a 34-kDa acidic, secreted phosphoprotein that was originally identified as a major component of the non-collagenous bone matrix expressed in both osteoblasts and osteoclasts. It has been found to be associated with bone strength and bone remodeling [10]. In addition, OPN has been shown to bind calcium ions [11], calcium oxalate crystal in urine, and HA crystal [12]. The prefix of the word "osteo" indicates that the protein is expressed in bone, although it is also expressed in other tissues. The suffix "pontin" is derived from "pons," the Latin word for bridge, and signifies OPN's role as a linking protein, and it was first identified in 1986 in osteoblasts [13].

Osteopontin is closely involved in the regulation of both physiological and pathological mineralization. In normal bone tissue, OPN is expressed by both osteoclasts and osteoblasts which are the cells responsible for bone remodeling. During normal bone mineralization, osteoclast derived osteopontin inhibits the formation of HA [14]. The protein clearly has a cell attachment capability for many cell types, including Osteoblasts, osteoclasts, and many transformed fibroblast lines [15]. The role of OPN in the regulation of bone metabolism has been suggested in many animal studies [16-18]. In the context of its involvement in a mineralization process, it is possible that high serum levels of OPN act directly on crystal nucleation or growth. Highly anionic OPN may limit the size of calcium-containing crystals by binding to existing crystals and preventing further growth [19].

Methods:

This is a cross-sectional study, conducted from June 2012 to April 2013, with fifty seven postmenopausal Iraqi women whose ages are fifty years old and over, categorized into two groups: healthy postmenopausal women (n=27), mean age of (58.44±4.54 year), and osteoporosis postmenopausal women (n=30), mean age (59.70±4.54 year).

The postmenopausal osteoporosis subjects selected from patients who visited Rheumatology and Rehabilitation Outpatient Clinic in Baghdad Teaching Hospital, and healthy postmenopausal females were selected from general population. The postmenopausal status was defined as cessation of menses for at least 1 year. All participants were interviewed and examined by physicians in a Rheumatology and Rehabilitation Outpatient Clinic. In addition, information was obtained from each subject about medication and history of previous medical or surgical diseases. Subjects with diabetes mellitus, high or low blood pressure, primary hyperparathyroidism, hyperthyroidism, rheumatoid arthritis, and hepatic or renal dysfunction; which might cause changes in bone metabolism, were excluded. None of the subjects were taking any drugs or hormones that affect bone metabolism; including sex hormones, glucocorticoids, warfarin, vitamin K, raloxifene and bisphosphonates. Body mass index (BMI), was calculated from the height and weight information by dividing the weight of the subject, in kilograms, per square of his height, in meter.

BMD in lumbar spine (anterior-posterior projection at T4-L4) of all subjects was measured by Dual Energy X-ray Absorptiometry, DEXA, using QDR-2000 system (Hologic Company, USA) which was controlled by computer with auto-position fixing, auto-detecting and auto-data manipulating. In practical operation, the subject lay down in the middle of the detecting bed, with her head leaving 3 cm to the bed top, her both hands on the sides of the body, and her two legs straightening and separating gently. The diagnostic criteria of osteoporosis proposed by World Health Organization (WHO) in 1994 were used, in which the T-score ≥-1.0 was considered as normal, -1<T-score<-2.5 considered osteopenia, and if T-score<-2.5 should be diagnosed as osteoporosis.

Superficial vein blood of 5 ml was collected from the elbow, while all women were at fasting state and after an overnight fast. Blood samples were allowed to clotting at room temperature for approximately thirty minutes. The serum was separated by centrifugation (3,000 rpm) for 10 minutes, and then the serum was isolated, within an hour of blood collection, and stored at −20°C for subsequent analyses, hemolyzed samples were excluded, and before analyses, samples were allowed to attain the room temperature.

All sera samples were analyzed for serum alkaline phosphatase, calcium and phosphorous by using spectrophotometric kit. Serum alkaline phosphatase was determined by colorimetric method for in vitro diagnostic measurement using kit manufactured by Biolabo, France, serum calcium was determined by colorimetric method for the quantitative in vitro diagnostic measurement using kit manufactured by Human, Germany, and serum phosphorous was determined by colorimetric method for the quantitative in vitro diagnostic measurement using kit manufactured by Human, Germany.

The samples were analyzed for bone turnover markers which include: bone formation marker; serum OC; which was measured by immunoassay (ELISA) using OC Enzyme Immunoassay Kit manufactured by Micro Vue,

San Diego, USA, and bone resorption marker; serum OPN; which was measured by enzyme immunoassay (ELISA) for the in vitro quantitative measurement of human OPN in serum, plasma, cell culture supernatants and urine (ELISA) using a kit supplied by Ray Bio, USA.

The collected data were analyzed statistically using student T-test to find the significant difference between means, and the correlation analysis to find the level of the correlation coefficient (r). P value less than 0.05 is considered as significant. All statistical analyses were performed with SPSS 16.0 software (SPSS, Chicago, IL, USA).

Results:

The characteristics of the subjects enrolled in the present study are shown in Table 1.

Table1: Clinical characteristic of study groups.

Parameters	Healthy Mean±SD	Osteoporotic Mean±SD	P Value
Number	27	30	-
Age (year)	58.4±3.7	59.7±4.5	0.0270
BMI (kg/m^2)	29.6±4.5	28.7±4.9	0.0570
BMD(gm/cm^2)	1.11±0.11	0.73±0.05	0.0001
T-score	-0.77±0.08	-3.03±0.42	0.0001
Serum Ca (mmol/L)	2.25±0.14	2.20±0.12	0.0790
Serum PO_4 (mmol/L)	1.19±0.17	1.14±0.16	0.1040
Serum ALP (U/L)	65.5±12.7	66.1±13.7	0.8990
Serum OC (ng/ml)	19.5-24.8	30.97±2.2	0.0001
Serum OPN (ng/ml)	15.5±0.59	21.1±2.44	0.0001

There were significant differences in age, BMD, T value, and the content of serum OC and serum OPN between osteoporotic postmenopausal group as compared with healthy postmenopausal group.

Serum OC levels, a marker of bone formation, were significantly higher in osteoporotic postmenopausal women as compared with healthy postmenopausal women (p=0.0001). Serum OPN, a marker of bone resorption, also showed a significant higher levels in osteoporotic postmenopausal women as compared with healthy postmenopausal women (p=0.0001).

Serum OC levels showed a significant positive correlation with age (r=0.807, p=0.0001) for the patients group, but there was no correlation with BMI (r=0.348, p=0.059) for the same group. Serum OPN levels also showed a significant positive correlation with age (r=0.533, p=0.002) for the patients group, but there was no correlation with BMI (r=0.385, p=0.350) for the same group.

In the present study, simple regression analysis revealed a strong and positive correlation between serum OC, a marker of bone formation, and serum OPN, a marker of bone resorption, in postmenopausal women (r=0.382, p=0.037), however, there was no correlation between these two markers in healthy postmenopausal women. These results suggest that the association between serum OC and serum OPN is not affected by menstrual state.

Discussion:

About 10% of the adult skeleton is remodeled each year, this turnover prevents fatigue damage and is important in maintaining calcium homeostasis. Bone loss results from imbalance between rates of resorption and formation [20].

Estrogen and androgen are the major sexual hormones in human body, and they participate in the formation and growth of bone, and play a very important role in maintaining mineral balance and bone balance in the body [21]. Estrogen and androgen exist in the blood of both men and women, and it is generally believed that estrogen may be related to the regulation of bone resorption, and androgen to the osteoblast differentiation [22].

People lacking of sex hormone are prone to have osteoporosis caused by bone loss [23].In addition, the ovary function of post-menopause women declines gradually near the age of menopause, testosterone is the most active androgen in women, and with the decrease of testosterone levels, the direct effect of androgen on maintaining bone quality will be lowered, and the amount of estrogen transformed from androgen will also be reduced, therefore, estrogen deficiency makes postmenopausal women at considerable risk for osteoporosis and related fractures.

Fracture risk is higher in older individuals, indicating quality factors in addition to BMD contribute to bone fragility and increased fracture risk. The strength of bone is dependent on four factors, which are the bone mineral density, bone structure, bone matrix, and accumulation of micro cracks [24].

The present study evaluates whether some biochemical markers of bone turnover could help to identify women with low BMD. The present study showed that elderly postmenopausal osteoporotic women have a significantly low BMD as compared with elderly healthy postmenopausal women; these results are in line with the findings of several researchers. Kitahara et.al, have reported that BMD declines approximately 2.5% annually during the first 5 years after menopause [25]. Vondracek et.al and Bischoff et.al have suggested that the accelerated bone loss occurs at a rate of 2-3% of total bone mass per year in women, which begins after menopause and may last from 6 to 10 years. As a result, estrogen deficiency makes postmenopausal women at considerable risk for osteoporosis and related fractures [26,27].

Furthermore, a significant low T-value in osteoporotic postmenopausal women was found comparing with healthy postmenopausal women, indicating bone loss with age and menopause, a rapid bone loss is commonly seen in elderly individuals and tend to be worsen with advancing age [28], however, the results of this study demonstrate non-significant correlation between BMI and the occurrence of osteoporosis in elderly postmenopausal women, which is similar to the results published by other researchers.

Moreover, bone loss leads to thinning, and sometimes perforation, of the trabecular plates, the comprise of the human skeleton which is about 80% cortical bone and 20% trabecular bone, which is more metabolically active, and the osteoporotic fractures tend to occur at sites comprising more than 50% trabecular bone. Trabecular perforation occurs where there is an increased in bone turnover; the resulting change in architecture leads to loss of strength disproportionate to the amount of bone lost. Therefore, it is necessary to diagnose it earlier for giving preventive treatment and for avoiding consequences [29]. This finding is not surprising because bone undergoes a continual process of resorption and formation in discrete bone remodeling units.

The analysis results of the present study did not find a significant correlation between the levels of serum calcium, phosphorous and alkaline phosphatase with the incident of osteoporosis, because bone formation markers are substances directly or indirectly produced by osteoblasts at each stage of osteoblast differentiation, these finding indicate that osteoporosis patients which included in this study didn't have metabolic bone disease other than osteoporosis, in other words, osteoporosis with or without pathological fracture is suspected with other bone disease such as osteomalacia which is characterized by a defect of mineralization of bone matrix; and most commonly attributable to impaired intake, production or metabolism of vitamin D. In most cases, the diagnosis of osteomalacia is suspected by the clinical history and by abnormalities in biochemical tests such as low values of serum and urinary calcium, serum phosphate and 25-hydroxyvitamin D, and high values for alkaline phosphatase and parathyroid hormone. This can mimic osteoporosis and can be excluded by biochemical tests. Similar results have been reported by Delmas et.al, which find that the reason of normal value of serum alkaline phosphatase levels in osteoporosis patients with or without fracture is that the serum pool of total ALP consists of several isomers from various tissues, including the liver, bone, intestine, spleen, kidneys, and placenta. In adults with normal liver function, about 50 % of total ALP activity in serum is from the liver, and 50 % is from bone [30].

OC is a specific non-collagenous protein synthesized and secreted by osteoblasts. Its main physiological functions are to maintain the normal bone mineralization rate, to inhibit the abnormal formation of HA crystal, and to be involved in bone remodeling through a negative feedback mechanism. Almost all circulating OC is produced by osteoblasts; therefore, the concentration of blood OC may specifically reflect the activity of osteoblasts [31]. Since, in the process of bone resorption, the OC in the matrix is able to be released into the blood, therefore, more precisely, OC is a specific indicator to evaluate the bone formation and bone turnover rate [32].

In the present experimental results, a significantly higher level of serum OC is found with the decrease of BMD for postmenopausal osteoporotic women as compared with the healthy subjects. Furthermore, a significant positive correlation between the levels of serum OC and age of elderly women is found to be related to the increase in bone formation as a consequence of increased bone resorption. The two processes are linked when bone remodeling is high and the results of this study reflect the physiological reparative response of bone involving osteoblast activation.

Some studies have shown significant correlation of serum OC with BMD in postmenopausal osteoporotic women, Vanita et.al, reported that the mean level of serum OC was found to be significantly elevated in postmenopausal osteoporotic women when compared with healthy postmenopausal women [33], Verit et al. found that OC is a promising marker of bone turnover useful in the diagnosis and follow-up of high turnover osteoporosis [34]. Moreover, Eastell et.al, have suggested that OC can be used as a sensitive indicator to evaluate the bone metabolism and the BMD in postmenopausal women [35].

Previous studies indicated that plasma OPN levels could be used as a biomarker for the early diagnosis of osteoporosis in postmenopausal women [36]. In this study, we demonstrated that there is a sequential increase in the plasma OPN level with the decay in BMD. This effect may imply that the increases of plasma OPN expression, which in turn increases the catabolic bone resorption and decreases the anabolic bone formation in postmenopausal women suggesting that OPN may play a pivotal role in net bone formation in menopausal women. In addition, the inhibition of osteoclastogenesis is one of the main mechanisms by which estrogen prevents bone loss in women of menopause age [37]. It is likely that estrogen may regulate either the production of or the target cell responsiveness to RANKL (receptor activator of NF-κB ligand), which activates the differentiation of cells of the monocytic lineage into mature osteoclasts [38]. Moreover, OPN stimulates CD44 (receptor for hyaluronic acid interact with other ligands) expression on the osteoclast surface, and works as an anchor to promote the attachment of osteoclasts for bone to bone resorption [39], CD44 is also required for osteoclast motility [36].

The findings of this study support the view that high OPN expression in postmenopausal women up-regulate osteoclasts motility and increase the ability of osteoclasts to resorb bone, causing high bone turnover in postmenopausal osteoporosis women.

Similar result was published by Tanaka et.al, who revealed that the OC and OPN of cortical bone are important predictors of fragility fracture and a decrease of non-collagenous protein synthesis by osteoblasts is possible cause of fragility fracture [24].

The present study demonstrated a positive correlation between the level of OPN and BMD in postmenopausal women, which is similar to the findings of Chang et.al, supporting the notion that OPN do play a role in human postmenopausal osteoporosis [36].

Conclusions:

This study showed an obvious increase in bone turnover markers in Iraqi postmenopausal osteoporotic women, which revealed that serum OC and OPN could be used as a biochemical markers for the diagnosis of osteoporosis in postmenopausal Iraqi women.

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