Original Article

Serum Osteocalcin and Serum Osteopontin Levels in Osteoporotic Postmenopausal Women with and without Vertebral Fractures

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Abstract:

Background: Osteoporosis is a progressive systemic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue spatially in postmenopausal women and its major complication fractures.

Objective: The aim of this study was to find out the significance of serum OC and serum OPN levels with the incidence of osteoporosis and its major complication (fractures).

Patients and Methods: Eighty-five postmenopausal women (PMW) whose ages were fifty years and over categorized into three groups: osteoporosis PMW without VFs (n=30), osteoporosis PMW with VFs (n=28), and healthy PMW (n=27). Sera samples were analyzed for alkaline phosphatase, calcium and phosphorous by using spectrophotometric kit. Serum OC and serum OPN levels were measured by ELISA kits.

Results: Bone Mineral Density (BMD) and T-score were significantly lower in osteoporotic PMW with and without VFs as compared with healthy PMW (p=0.0001, p=0.0001, respectively). Serum OC levels and serum OPN levels were elevated significantly in osteoporotic PMW with and without VFs as compared with healthy PMW (p=0.0001, p=0.0001, respectively), the levels of serum OC and OPN showed a significant positive correlation with age in osteoporotic PMW. There is a significant positive correlation between serum OC levels and serum OPN levels in osteoporotic PMW and non-significant correlation was found in healthy PMW.

Conclusion: The levels of serum OC and OPN can be used as a biochemical indicator in the diagnosis of postmenopausal osteoporotic women.

Keywords: Postmenopausal, Osteoporosis, Vertebral Fractures, Osteocalcin, Osteopontin.

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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). The development of osteoporosis in elderly women is related to the deficiency of estrogen which prevents the absorption and utilization of calcium. Menopause and ageing is associated with accelerated loss of cortical bone (2). Bone loss occurs when the balance between formation and resorption is upset and the resorption is excessive resulting in a negative remodeling balance (3). Biochemical markers of bone turnover reflect the status of bone metabolism in various processes coupled with bone resorption and formation (4). Individuals with increased bone turnover markers lose bone at a faster rate than subjects with normal or low bone turnover markers (5).Osteocalcin (OC) is a small protein, 49 amino acids long. It is includes 3 residues of gamma- carboxyglutamic acid. It has a negatively charged surface that places five calcium ions in positions surface that places five calcium ions in positions complementary to those in hydroxyapatite (HA), an important mineral component of bone. Osteocalcin is the most abundant non-collagenous protein in bone. 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, formula $Ca_5 (PO_4)_3OH$) (6). 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 (7).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 (80. It has been found to be associated with bone strength and bone remodeling (9). In addition, OPN has been shown to bind calcium ions (10), calcium oxalate crystal in urine, and HA crystal (11). Osteopontin is closely involved in the regulation of both physiological and pathological mineralization. During

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normal bone mineralization, osteoclast derived osteopontin inhibits the formation of HA (12). The role of OPN in the regulation of bone metabolism has been suggested in many animal studies (13). Highly anionic OPN may limit the size of calcium-containing crystals by binding to existing crystals and preventing further growth (14).The aim of this study was to investigate the significance of serum osteocalcin and serum osteopontin, in the diagnoses of osteoporosis in postmenopausal women with and without history of vertebral fractures.

Patients and Methods:

This study was conducted from July 2012 to March 2013 with eighty five (85) PMW whose ages were fifty years and over, categorized into three groups: healthy PMW (n=27), mean age of (58.44±4.54 year), osteoporosis PMW without VFs (n=30), mean age (59.70±4.54 year), and osteoporosis PMW with VFs (n=28), mean age (61.78±5.25 year).The osteoporotic PMW subjects with and without VFs were 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 one year. All participants were interviewed and examined by physicians in a Rheumatology and Rehabilitation Outpatient Clinic. In addition, information was obtained from each woman about medication and history of previous medical or surgical diseases. Women with diabetes mellitus, primary hyperparathyroidism, hyperthyroidism, rheumatoid arthritis, and hepatic or renal dysfunction were excluded. None of the patients 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 her height, in meter. Bone Mineral Density Measurement: Bone Mineral Density in lumbar spine (anterior-posterior projection at T4–L4) of all women was measured by Dual Energy X-ray Absorptiometry, DEXA, using QDR-2000 system (Hologic Company, USA) which was controlled by computer. 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. Vertebral Assessment: Vertebral fractures were ascertained by review of radiograph reports for all subjects in this study. Therefore, all the subjects underwent a lateral X-ray of the spine. Anterior, middle, and posterior heights of vertebrae T4 to L4 were measured on lateral radiographs by using a ruler. According to Kleerkoper's method for diagnosis of vertebral fracture. Blood Samples: Superficial vein blood of 5 ml was collected, while all women were at fasting state and after an overnight fast. Blood samples were allowed to clot 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, hemolysed samples were excluded, and before analyses, samples were allowed to attain the room temperature.

Biochemical Tests: All sera samples were analyzed for 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.

Bone Turnover Markers Measurements: The samples were analyzed for bone turnover markers which include: bone formation marker; serum OC; which was measured by enzyme-linked immunosorbent assay (ELISA) using OC Enzyme Immunoassay Kit manufactured by MicroVue, San Diego, USA, and bone resorption marker; serum OPN; which also was measured by 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.

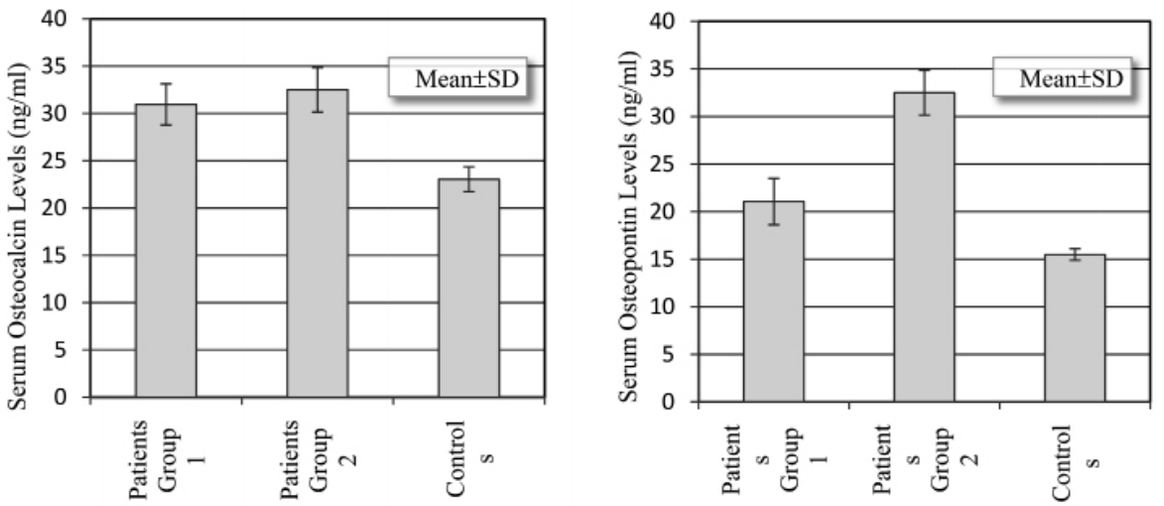
Statistical Analysis: The collected data were analyzed statistically using one way ANOVA to find the significant difference between means, and the correlation analysis to find the level of the correlation coefficient (r). p value less than 0.05 is considered as significant. All statistical analyses were performed with SPSS software (SPSS version 16.0, Chicago, IL, USA).

Results:

The characteristics of all the subjects which participate in this study are shown in Table 1. Henceforward we will use "Patients Group 1" for PMW without VFs, "Patients Group 2" will refer to PMW with VFs, and "Controls" will represent the healthy PMW. There were significant differences in age, BMD, T-score, and the content of serum OC and serum OPN between Patients Group 1 and Patients Group 2 as compared with the Controls.The comparison of bone turnover markers in the three studied groups is shown in Fig. 1. Fig. 1a shows that serum OC levels, a marker of bone formation, were significantly higher in osteoporotic PMW with and without VFs as compared with healthy PMW (p=0.0001). Serum OC levels is higher in Patients Group 2 as compared with Patients Group 1. Serum OPN levels, a marker of bone resorption, showed a significant higher levels in osteoporotic PMW with and without fracture as compared with healthy PMW (p=0.0001). Similarly, serum OPN levels is much higher in Patients Group 2 as compared with Patients Group 1.

Table (1): Clinical characteristic of Patients Group 1, Patients Group 2, and Controls

Parameters	Controls	Patients Group 1	Patients Group 2	p value
N	27	30	28	
Age (year)	58.44±3.74	59.70±4.54	61.78±5.25	0.0270
BMI (kg/m^2)	29.64±4.49	28.65±4.93	26.51±3.97	0.0570
BMD(gm/cm^2)	1.10±0.11	0.73±0.05	0.63±0.08	0.0001
T-score	-0.77±0.08	-3.03±0.42	-4.21±0.89	0.0001
Serum Calcium (mmol/L)	2.25±0.14	2.20±0.12	2.18±0.12	0.0790
Serum Phosphorus (mmol/L)	1.19±0.17	1.14±0.16	1.09±0.14	0.1040
Serum ALP (U/L)	65.52±12.7	66.07±13.67	67.07±11.45	0.8990
Serum OC levels (ng/ml)	19.5-24.8	30.97±2.17	32.50±2.35	0.0001
Serum OPN levels (ng/ml)	15.496±0.596	21.065±2.439	32.504±2.352	0.0001



(a) (b)

Fig. 1 Comparison of the results of serum OC and OPN levels in the three studied groups, (a) serum OC levels, (b) serum OPN levels

Serum OC levels showed a significant positive correlation with age (r=0.807, p=0.0001) for Patients Group 1 but there was non-significant 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 Patients Group 1 but also there was non-significant correlation with BMI (r=0.385, p=0.350) for the same group.

There was a significant positive correlation between serum OC levels and age (r=0.663, p=0.0001) for Patients Group 2, and there was no correlation for serum OC levels with BMI (r=0.110, p=0.576) for Patients Group 2. There is a significant positive correlation between serum OPN levels and age (r=0.653, p=0.0001) for Patients Group 2 but there was non-significant correlation with BMI (r=-0.236, p=0.227) for the same group. Serum OC levels for Controls showed a non-significant correlation with age (r=0.373, p=0.055), and BMI (r=0.149, p=0.457). Serum OPN levels also showed a non-significant correlation with age (r=-0.080, p=0.691) and BMI (r=-0.020, p=0.920) for the same group. Simple regression analysis revealed that serum OPN levels were significantly and positively correlated with serum OC levels in Patients Group 1 and 2; r=0.382 and p=0.037 for Patients Group 1, and r=0.412 and p=0.029 for Patients Group 2, however, simple regression analysis showed that serum OPN levels were non-significantly correlated with serum OC levels in control subjects; r=-0.095 and p=0.636.

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 an imbalance between rates of resorption and formation (15). 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 (16). 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 (17). People lacking of sex hormone are prone to have osteoporosis caused by bone loss (18). 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 microcracks (19). The present study evaluates whether some biochemical markers of bone turnover could help to identify women with low BMD. Moreover it showed that elderly postmenopausal osteoporotic women with and without fracture 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 (20). Also Vondracek et al. and Bischoff et al. have been suggesting that the accelerated bone loss occurs at a rate of 2% to 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 (21). Furthermore, a significant low T-score in osteoporotic postmenopausal women with and without vertebral fracture 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 (22). Moreover 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 (22). The result of this study also demonstrate that osteoporotic postmenopausal women with fracture have significantly low values of BMD and T-score as compared with osteoporotic postmenopausal women without fracture which suggest that the aging population is more prone to osteoporotic fracture. The reason of that is 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. Bone loss leads to thinning, and sometimes perforation, of the trabecular plates. 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 (23). The statistical analysis of the results did not show a significant correlation between the levels of serum calcium, phosphorous and alkaline phosphatase with the incident of osteoporosis with or without vertebral fracture, because bone formation markers are substances directly or indirectly produced by osteoblasts at each stage of osteoblast differentiation. These finding indicate that osteoporosis patients with or without fracture included in this study didn't have metabolic bone disease other than osteoporosis. Similar results have been reported by Delmas et al., which showed 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 isozymes 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 (24).In the present experimental results, a significantly higher level of OC is found with the decrease of BMD, for postmenopausal osteoporotic women with and without fracture, 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, Verit et al. found that OC is a promising marker of bone turnover useful in the diagnosis and follow-up of high turnover osteoporosis (25). Similar observations were reported by other studies, e.g. Eastell et al. have been suggested that OC can be used as a sensitive indicator to evaluate the bone metabolism and the BMD in postmenopausal women (26). Previous studies indicated that serum OPN levels could be used as a biomarker for the early diagnosis of osteoporosis in postmenopausal women (27). In this study, we demonstrated that there is a sequential increase in the serum 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 suggest 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 (28). 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 (29). 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 (29). 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 turnover postmenopausal osteoporosis. Similar result was subsequently published by other researchers, S. Tanaka et al. 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. OPN suppresses the growth of calcium phosphate crystals, and an OC deficit does not result in reduced calcification or bone formation (20). The present study demonstrated a positive correlation between the level of OPN and BMD in postmenopausal women which is similar to Chang et al, findings which support the view that OPN does play a role in human postmenopausal osteoporosis (27).

Conclusion:

This study showed an obvious increase in bone turnover markers in osteoporotic PMW. The levels of serum OC is higher in osteoporotic PMW with vertebral fractures as compared with those without vertebral fractures. The levels of serum OPN is significantly higher in osteoporotic PMW with vertebral fractures as compared with those without vertebral fractures. This study revealed that serum OC and OPN could be used as a biochemical markers for the diagnosis of osteoporosis in postmenopausal women.

Authors Contributions:

Nawar S Mohammed: Collecting samples, analysis of data, and writing the manuscript

Kisma M Turki: Study design and revision of the writing

Mohammed H Munshed: Diagnosis and sampling

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