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Original Research Article

The Values of Osteopontin and Bone Specific Alkaline Phosphatase in Diagnosis of Osteoporosis. AComparative Study

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Abstract

Dual-energy X-ray absorptiometry (DXA) is commonly used for diagnosis of osteoporosis, but it has its limitations. Bone turnover markers have advantages over DXA as they are cheap, non-invasive and can detect changes in bone turnover rates earlier. However, they do have disadvantages, particularly high within- and between-patient variability. The ability of bone turnover markers to predict of osteoporosis has yet to be established. The objective of this study is to assess the use of bone markers for diagnosis of osteoporosis in postmenopausal women and compare the results with those obtained by DXA method. This case- control study was conducted between October 2012 and April 2014. Forty-four postmenopausal osteoporotic women and forty-four healthy women as a control group, aged between 50-65 years old were involved in this study. They were recruited from the Al-Yarmouk Teaching Hospital, Baghdad, Iraq, and selected on the basis of inclusion and exclusion criteria. The physical characteristics were assessed using anthropometric measurements including height, weight, and body mass index (BMI). Bone mineral density (BMD) of lumbar and hipwere measured by the dual-energy X-ray absorptiometry (DXA) instrument. Serum Calcium and Phosphorous were measured using spectrophotometric kit while serum levels of osteopontin and bone specific alkaline phosphatase were measured by Enzyme-Link Immune Sorbent Assay (ELISA).Receiver operator characteristic (ROC) curves were constructed to plot sensitivity against specificity of lumbar and hip BMD, OPN and BAP levels as diagnosis tests for osteoporosis. The areas under the ROC curves (AUC) were calculated and compared with the AUC (0.5) of the non-diagnostic test (the line with slope of 1).

The osteoporotic postmenopausal women had higher mean serum OPN and BAP levels compared to the healthy women (16.41 ± 6.33 vs 7.13 ± 5.88 ng/mL; $P < 0.01$); (47.08 ± 36.47 vs 24.42 ± 16.86; $P < 0.01$), respectively. When using a OPN concentration of 13.25 ng/ml as a cut off value for the diagnosis of osteoporosis sensitivity was 81.8%, specificity 86.4% and when using a BAP concentration of 31.05 ng/ml as a cut off value sensitivity was 81.8%, specificity 86.4%, Where is the sensitivity of lumbar and hipBMD was 100%, specificity 100% as a cut off value 0.925 g/cm^{2}and 0.785 g/cm^{2}respectively. There were no significant differences in mean values of $Ca^{+2}$and P ($p>0.05$).

The DXA is a more valid and reliable tool to utilize in diagnosis of osteoporosisin postmenopausal women than the measurement of serum OPN and BAP level.

Key words: Osteoporosis,Bone mineral density, Osteopontin,Post menopause, Bone specific alkaline phosphatase.

الأوستيوبونتين وأنزيم الفوسفات القاعدي العظمي في تشخيص مرض هشاشة العظام لدى المريضات المصابات بعد سن اليأس

الخلاصة

يستخدم الدكسا (DXA) عادة لتشخيص هشاشة العظام، ولكن له حدوده. فالمؤشرات الحيوية لسرعة تغير العظم ممكن ان تكون لها مزايا أكثر من الدكسا كونها رخيصة، لا تحتاج الى تداخل مع المريض ويمكن الكشف عن المرض مبكرا من خلالها. ومع ذلك, لم يتم التنبؤ من خلالها

عن مرض هشاشة العظام بسبب تغيراتها في نفس المريض ومن مريض الى آخر.كان الهدف من هذه الدراسةتقييم استخدام علامات العظم لتشخيص هشاشة العظام في النساء بعد سن اليأس ومقارنة النتائج مع تلك التي تم الحصول عليها عن طريق إستخدام تقنية الدكسا DXA. بين ٥٠ الى ٦٥ سنة قسمت الى مجموعتين, ٤٤أمرأة يعانين من هشاشة العظام و ٤٤ امرأة سليمة من مرض هشاشة العظام ، .وقد تم اختيار المرضى على أساس معايير الاشتمال و الإقصاء . تم الاختيار في إستشارية المفاصل في مستشفى اليرموك التعليمي، بغداد، العراق، بين أكتوبر٢٠١٢وأبريل ٢٠١٤. تم تقييم الخصائص الفيزيائية باستخدام قياسات الجسم البشري بما في ذلك الطول والوزن، ومؤشر كتلة الجسم (BMI)، تم قياس كثافة العظام من الفقرات القطنية والورك بواسطة الدكسا(DXA). وتم قياس الأوستيوبونتين(ONP)وإنزيم الفوسفات القاعدي العظمي(BAP)في الدم بواسطة تقنية الأيلايزا (ELISA). أخذت عينات عشوائية للكالسيوم والفوسفات وأنزيم الفوسفات القاعدي (ALP)في الدم،وتم قياسها باستخدام مقياس الطيف الضوئي. بينت نتائج الدراسة أن مستوى كثافة العظم (بإستخدام جهاز الدكسا) في الفقرات القطنية والورك أقل بكثير في مجموعة النساء بعد سن اليأس المصابات بهشاشة العظام مقارنة مع مجموعة النساء السليمات.باستخدام منحني ال (ROC) Receiver Operator Characteristic لقياس مستوى كثافة العظم بإستخدام جهاز الدكسا ,وعند استخدام مستوى كثافة العظم بتركيز (g/cm^2)0.925للفقرات القطنية وتركيز (g/cm²) 0.785 للورك كمستوى فاصل (Cut–off value) كانت الانتقائية 100%وبحساسية مقدارها 100% وكانت القيمة التنبؤية الايجابية 100% والقيمة التنبؤية السلبية 100% لكليهما. معدل مستوى مصل الدم للأوستيوبونتين المصلي(OPN) كانت اعلى بشكل ملحوظ في النساء بعد سن اليأس المصابات بهشاشة العظام بالمقارنة مع مستوياتها في دم النساء السليمات بعد سن اليأس.باستخدام منحني ال (ROC) Receiver Operator Characteristic وعند استخدام مستوى الاوستيوبونتين(OPN) بتركيز (ng/ml) 13.25كمستوى فاصل (Cut–off value) كانت الانتقائية86.4%وبحساسية مقدارها 81.8% وكانت القيمة التنبؤية الايجابية 85.71% والقيمة التنبئية السلبية 82.61%. إنزيم الفوسفات القاعدي العظمي (BAP) كانت اعلى بشكل ملحوظ في النساء بعد سن اليأس المصابات بهشاشة العظام بالمقارنة مع مستوياتها في دم النساء السليمات بعد سن اليأس. باستخدام منحني ال (ROC) Receiver Operator Characteristic وعند استخدام مستوى إنزيم الفوسفات القاعدي العظمي(BAP) بتركيز(ng/ml) 31.05 كمستوى فاصل (Cut–off value) كانت الانتقائية70.5%وبحساسية مقدارها 61.4% وكانت القيمة التنبؤية الايجابية 67.5% والقيمة التنبؤية السلبية 64.58%.تشير النتائج من الدراسة الحالية أن الدكسا (DXA) هو أداة أكثر دقة ووثوق للاستفادة في تشخيص هشاشة العظام النساء بعد سن اليأس من قياس الأوستيوبونتين (OPN)ومستوى إنزيم الفوسفات القاعدي العظمي(BAP).

الكلمات المفتاحية : هشاشة العظام ، كثافة العظم ، الاوستيوبونتين.

Introduction

Bone is a dynamic organ and remodels itself throughout life by process called bone remodeling which involves removal of old bone from one surface of bone and forms new bone to prevent accumulation of bone micro damage. The remodeling activity varies in different types of bone, with cancellous bone having a higher turnover rate than cortical bone[1].There is strong evidence for an association between a high rate of bone remodeling and osteoporosis[2]. For bone to remain healthy the process of bone breaks down and bone formations have to remain balanced[3]. Osteoporosis occurs if this process of remodeling is interrupted[4]. Osteoporosis can develop when there is a net decrease in bone formation. This may be due to either increased bone resorption, decreased bone formation, or a combination of both. The result isbone loss begins and bone cortex and trabeculae become thinner and perforated resulting in increased cortical porosity and a destruction of trabeculae, which account for age- dependent bone loss[5].

Osteoporosis is a systemic skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture risk[6].Osteoporosis (OP) has become an alarming health problem through the entire world and about 200 million people in the world are under the threat of this deleterious health problem [7].According to World Health Organization (WHO) estimates the number of postmenopausal women in the year 2025 will be around 60–70 million[8].

Measurement of bone mineral density (BMD) is gold standard for diagnosis of osteoporosis which is measured by dual energy X-ray absorptiometry (DXA) by

determining the absorption of two beams of photons at two different energies. It is painless, a bit like having an x ray, but with much less exposure to radiation.Limitations with DXA technique are inclusion of artifacts such as osteophytes, surgical implants, vertebral fractures and osteoarthritis of the spine leading to hypertrophic bony changes that can alter the approximation of bone density which falsely elevates the BMD. For these patients, measurement of the biochemical markers in blood or urine, which define either markers of osteoblastic or osteoclastic function,which is called bone turnover markers (BTM), may be better.

Osteopontin (OPN) one of the bone resorption markers, it is a phosphorylated noncollagenous bone matrix glycoprotein produced by osteoblasts and osteoclasts.It is a multifunctional protein, highly expressed in bone.It plays important roles in bone resorption by facilitates osteoclast attachment to the bone matrix[9,10].

Osteoclasts express αvβ3 integrin, which is a receptor for osteopontin.Yamate et al. [11] reported that the expression of osteopontin by osteoclast and osteoblast progenitors in murine bone marrow increased after ovariectomy. It is speculated that the bone loss observed may be related to the requirement of efficient signalling through the αvβ3 integrin for optimal osteoclast function. So if osteopontin is deficient, as it is in knock-out mice, there is no high-affinity interaction between osteopontin and αvβ3 integrin and therefore less bone resorption activity by osteoclasts.

Bone alkaline phosphatase is the most widely used marker of bone formation, and it is actively excreted by osteoblasts. Bone alkaline phosphatase may increase local phosphorus concentrations, remove phosphate-containing inhibitors of hydroxyapatite crystal growth, or modify phosphoproteins to control their ability to act as nucleators.In osteoporosis, BAP may be moderately increased due to the generalized increased bone turnover. Raised BAP is a marker of osteoblast activity and hence bone formation[12].

The aim of this study was to find new biochemical markers for diagnosis of osteoporosis, may be better than the measurement of BMD which is until now the gold standard for diagnosis of osteoporosis by DXA method.

Materials and Methods

The study was conducted at department of Chemistry and Biochemistry, College of Medicine, Universityof Al-Nahrainand Al-Yarmouk Teaching Hospital from October 2012 to April 2014. A total of eighty-eight postmenopausal women aged 50-65 years old were participated in the study. Exclusion criteria included patients who were bedridden, using steroids, or if they had such conditions affecting bone metabolism as diseases of the kidney, liver, parathyroid, or thyroid, diabetes mellitus, hyperprolactinemia, oophorec-tomy, rheumatoid arthritis, ankylosing spondylitis, malabsorption syndromes, malignant tumors, hematologic diseases, previous pathological fractures, hypertension, coronary artery disease, angiopathy, myocardial infarction, cerebral infarction, and infectious disease. If the subjects had received treatment with glucocorticoids, estrogens, thyroid hormone, parathyroid hormone, fluoride, bisphosphonate, calcitonin, thiazide diuretics, barbiturates, anti-seizure medication, vitamin D and calcium-containing drugs.

Body height and weight were measured using a stadiometer and a standardized balance-beam scale, respectively.

The study was approved by the local ethics committee of the College of Medicine/ University of Al-Nahrain.

Among the postmenopausal women, 44 were considered osteoporotic according to the WHO criterion: Bone mineral density (BMD) more than 2.5 SD below the normal adult mean based on the established reference databases. The other 44 women whose BMD was less than 1.0 SD below the normal were included into the normal group.

Bone mineral density (BMD) of the lumbar spine (first to fourth lumbar vertebrae), and the left femoral neck, was determined by dual energy X-ray absorptiometry (DXA), using DEXXUM3 system (Osteosys Co., Ltd., Korea) at Al-Yarmouk Teaching Hospital, Baghdad, Iraq. All BMD results were expressed as g/cm^2. According to definitions of diagnostic categories suggested by a WHO study group in 1994, the normal category has a BMD within 1 SD of the reference mean (young adults). In osteopenia the BMD value is more than 1 SD below the reference mean, whereas in osteoporosis the BMD is 2.5 SD or more below the reference mean. Expressed as T-scores:

Normal if T-score > -1;
osteopenia if -2.5 <Tscore< -1;
osteoporosis if T-score < -2.5. (WHO 1994)[13]

Blood samples were taken at the morning with no regard to meals and without tourniquet. Five milliliters (5 ml) of venous blood were collected from all the participants. The blood sample was collected in a plain tube and centerfuged for 15 minutes after being allowed to clot at room temperature for 30 minutes. The serum separated and stored at −20°C until time of analyses, hemolyzed samples were excluded. The local ethics committee of our college approved the study. All patients were informed on the study design and informed consent was obtained from each patient.

Statistical analysis

All values were expressed as mean ± standard deviation (mean ± SD). All Statistical analysis was performed using Social package statistical system (SPSS version 18). Group comparison was performed using students t-test. Pearson correlation coefficient was used to determine the correlation between two different variables. P value < 0.01 was considered statistically significant.

Results

The characteristics of the subjects enrolled in the present study were shown in (Table 1).There was highly significant difference in meanvalue of age between osteoporotic group and normal group, the postmenopausal women were in average 10 years older (P < 0.01). There are no significant difference (P > 0.05) in meanvalue of weight, height and BMI between both groups of osteoporotic patients and control group.

Table 1 : The characteristics of the studied subjects

Characteristics	Osteoporotic women (mean±SD) (n=44)	Control (mean±SD) (n=44)	P value
Age(years)	62.63±9.52	53.72±7.09	<0.01
BMI(kg/m^2)	31.84±4.47	30.81±3.54	<0.05
Weight(kg)	76.93±12.27	76.04±9.88	>0.05
Height(cm)	155.25±7.15	156.25±6.46	>0.05

As shown in (Table 2) there was a highly significant increase in serumosteopontin concentration in osteoporotic group (P < 0.01), when compared with its concentration in healthy control mean value. The mean serum BAP value was significantly higher in women with osteoporosis compared with the group of healthy women in which indicates a high rate of bone formation to the first group.Calcium and Phosphorous were shown to have a little variation even though it was statistically not significant (P>0.05) between both groups of osteoporotic patients and control group.

Table 2 : Comparison of biochemical parameters between patient group and control group by unpaired student's t-test

Parameters	Patients (n=44) Mean±SD	Control (n=44) Mean±SD	P value
Osteopontin (ng/ml)	16.41±6.33	7.13±5.88	**<0.01****
BAP # (ng/ml)	47.08±36.47	24.42±16.86	**<0.01***
S. Calcium (mg/dL)	9.16±0.66	9.0±0.68	>0.05
S. Phosphorous (mg/dL)	3.55±0.58	3.34±0.61	>0.05

** P value <0.01 is statistically highly significant.
* P value < 0.05 is statistically significant.
# Bone alkaline phosphatase

(Table 3) showed the mean values of BMD, expressed in g/cm ^{2} of lumber and hip of osteoporotic group and healthy control group. According to the WHO criteria the T-score of lumbar and hip and the BMD were normal in the control group. The corresponding values to the women with osteoporosis were much lower. The mean values of all these data shows highly significant differences (P < 0.01) between both group.

Table 3 : Comparison of radiological parameters between patient group and control group by unpaired students t-test

Parameters	Patients (n=44) Mean±SD	Control (n=44) Mean±SD	P value
Lumbar BMD (g/cm²)	0.73±0.06	1.13±0.08	**<0.01****
Lumbar T-score	-3.16±0.53	0.18±0.65	**<0.01****
Hip BMD (g/cm²)	0.5±0.1	0.97±0.09	**<0.01****
Hip T-score	-3.24±0.5	0.39±0.73	**<0.01****

** P value < 0.01 is statistically highly significant.

Receiver operator characteristic curve of lumbar BMD showed that the cut off for BMD was 0.925 g/cm², which was statistically significant (p<0.01) and had the highest accuracy, with sensitivity of 100 % , specificity of 100% , the positive predictive value was 100% and the negative predictive value was 100% as shown in (Table 4, Fig 1).

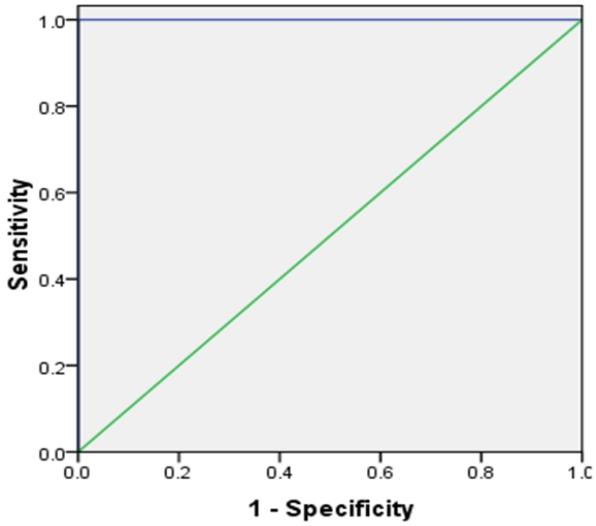

Figure 1 : ROC curve for lumbar BMD between control and osteoporotic group

Table 4 : Validity Indication of lumbar and hip BMD, serum osteopontin and serum BAPin prediction of osteoporosis

	Cutoff value	Specificity	Sensitivity	Area under curve	PPV	NPV	Efficiency	P value
lumbar BMD	0.925 (g/cm²)	100%	100%	1.0	100%	100%	100%	< 0.01
hip BMD	0.785 (g/cm²)	100%	100%	1.0	100%	100%	100%	< 0.01
serum osteopontin	13.25 (ng/ml)	86.4%	81.8%	0.846	85.71%	82.61%	84.09%	< 0.001
serum BAP*	31.05 (ng/ml)	70.5%	61.4%	0.721	67.5%	64.58%	65.91%	< 0.001

PPV Positive predictive value **NPV Negative predictive value**

***Bone specific alkaline phosphatase**

(Table 4, Fig 2) showed the receiver operator characteristic curve which demonstrated that the cutoff for hip BMD of 0.785 g/cm² had the highest accuracy, with sensitivity of 100%, and specificity of 100%, the positive predictive value was 100% and the negative predictive value was 100%.

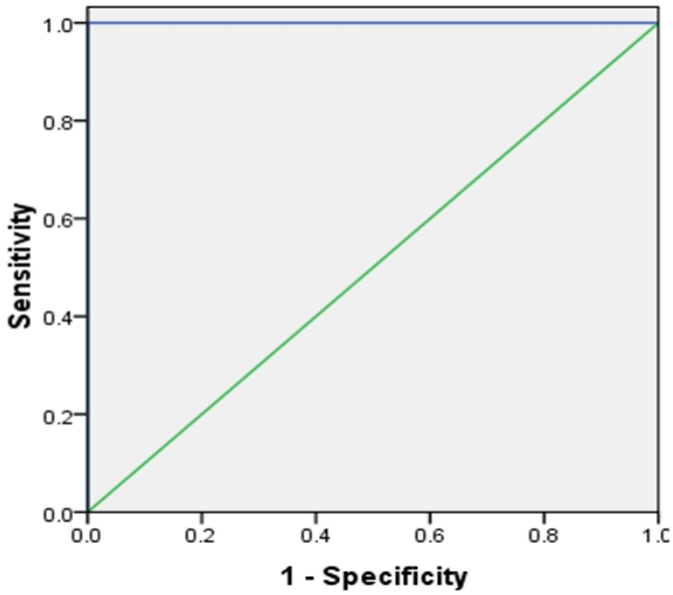

Figure 2 : ROC curve for hip BMD between control and osteoporotic group

The receiver operator characteristic curve demonstrated the optimal cut off for osteopontin value was 13.25 ng/ml, which was statistically significant ($p<0.01$), with sensitivity of 81.8 %, specificity of 86.4 %, the positive predictive value was 85.71% and the negative predictive value was 82.61% as shown in (Table 4, Fig 3).

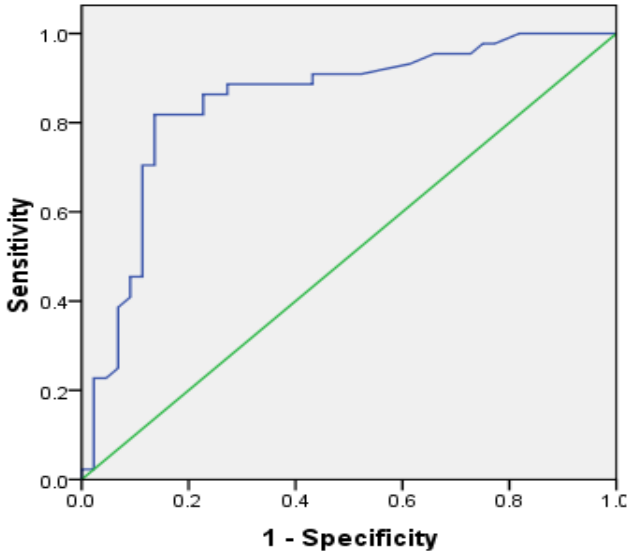

Figure 3 : ROC curve for serum osteopontin between control and osteoporotic group

The receiver operator characteristic analysis showed that the cutoff value for BAP was 31.05 ng/ml, which statistically significant ($p<0.01$), with sensitivity of 61.4%, specificity of 70.5%,the positive predictive value was 67.5% and the negative predictive value was 64.58% as shown in (Table 4, Fig 4).

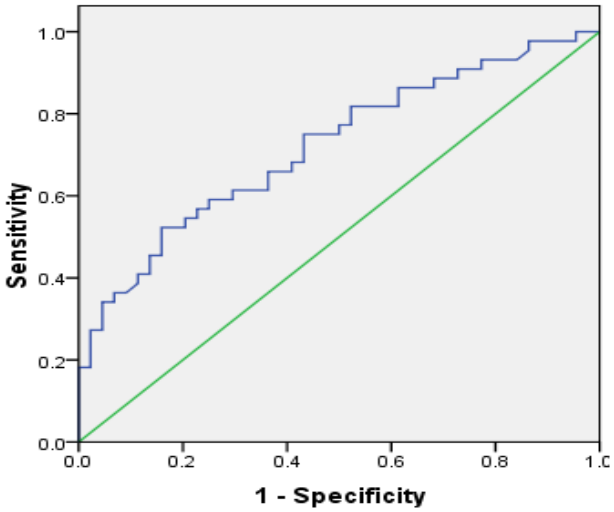

Figure 4 : ROC curve for BAP between control and osteoporotic group

Discussion

Bone mineral density (BMD) testing is the most effective way to determine an individual's bone health. BMD tests have the ability to: assess fracture risk[14], detect low bone density before a fracture occurs, confirm a diagnosis of osteoporosis if an individual has already experienced one or more fractures, predict the chances of future fractures occurring, and track an individual's rate of bone loss. The determination of bone mineral content and bone mineral density is essential in the investigation of an individual's calcium metabolism and associated diseases[15]. The density of each individual's bones is dependent upon the amount of stress placed on the bone, as well as the nutritional intake of the individual. When bone mineral density (BMD) measurement by itself does not provide a clear answer. The combined use of BMD measurement and bone markers is likely to improve the assessment of the risk of fractures in those cases[16-18].

In this study, the BMD of lumbar and hip of the healthy women have a significantly high BMD as compared with osteoporotic women (Table 3), this results are in line with the findings of several researchers[19,20].Jarupanich [21] has reported variation in BMD values in women of different menstrual status and low BMD values which leads to the diagnosis of osteopenia and osteoporosis.

Furthermore, a significant high T-score in healthy women was found comparing with osteoporotic women (Table 3), indicating bone loss with age and menopause. It's very interesting that, by means of ROC curve analysis (Table 4, Fig 1&2), the measurement of BMD was found to be a reliable test for discriminating osteoporosis person from normal. ROC analysis showed that the cutoff for lumbar BMD was 0.925 and for hip BMD was 0.785, both had the highest accuracy, with sensitivity of 100%, and specificity of 100%.

In recent years cellular components of the bone matrix have been identified and categorized as either markers of bone formation or resorption, which is called bone turnover markers (BTM), that can

be measured in blood or urine. Various biochemical markers are now available that allow a specific and sensitive assessment of the rate of bone formation and bone resorption of the skeleton[22]. OPN is one of the bone resorption markers, of the most, it is one of abundant non-collagenous proteins in bone. OPN is found in most if not all body fluids, perhaps acting to prevent mineral precipitation from these solutions, it does not appear to play an essential role in the normal processes of soft tissue differentiation or homeostasis. Despite the exact roles of OPN in bone are not fully understood, many studies established that OPN is an inhibitor of calcification[23,24].The expression of OPN has been reported to be regulated by mechanical stress, both in vitro and in vivo, and the ability of this protein to influence bone homeostasis through the inhibition of mineral deposition is well known[25,26]. Bones from OPN-knockout mice have a greater mineral content and contain larger hydroxyapatite crystals than wild type animals[27]. It is probable that osteocytes elaborate OPN to inhibit the growth of hydroxyapatite in the wall of their lacuna and in the canaliculi to avoid "suffocation" due to secondary mineralization. OPN is known to be a major ligand for CD44 on bone cells, which is important in mediating osteoclast recruitment and function [28-30] and also required for osteoclast motility[29].As elegantly shown by Chellaiah et al. the reduction in functionality of OPN-deficient osteoclasts can be attributed to decreased motility which was caused by a lack of cell surface expression of CD44[28]. Susan et al reported that calcification of the matrix appeared unaffected by the absence of OPN[30].

In this study estimation of OPN with a cut-off value of 13.25 ng/ml served as sensitivity 81.8%, specificity 86.4% (table 4). Also the most striking finding of the present study was the appearance of low concentration of serum osteopontin of the healthy postmenopausal women of this study compared with that for osteoporotic postmenopausal women (Table 2), and this is in agreement with those reported by others previously[31,32]. That indicate that high OPN levels were associated with osteoporosis in postmenopausal women and OPN is a risk factor for osteoporosis. Chang et al [31], Cho et al. [32] andFodor et al. [33] found that the high serum levels of OPN were associated with low BMD and increased levels of bone turnover markers. In the osteoporotic and control groups of this study, there was no correlation between osteopontin and any other parameters, and this is in consistent with Cho et al. [32] who also failed to show any correlation between serum OPN levels and clinical parameters with the exception of some BMD values in post-menopausal women. The possible explanation for this discrepancy could be attributed to the small sample size also the previous studies have shown that markers of bone resorption are more affected by circadian variation and feeding than formation markers[34].

For all demographic groups, the rates of osteoporosis increase with age[35]. Aging is associated with significant bone loss in women and in men[36]. Our study shows a significant difference in mean value (Mean ±SD) in age between patients group and controls (Table 1), which indicating that the age is an important factor for the prevalence of osteoporosis. This finding agreed with many studies that explain that the age also means longer total exposure to chronic oxidant stress and inflammation, both of which contribute to development of osteoporosis[37-40].

The present study showed no significant differences in calcium and phosphorous between osteoporotic women and healthy women; this is because that the level of calcium in the blood is mainly controlled by parathyroid hormone and calcitonin.

When using BAP concentration of 31.05 ng/ml as a cut off value for the diagnosis of osteoporotic women from control

group, sensitivity was 61.4%, specificity was 70.5% (Table 4, Fig 4).In this investigation significant elevated values of BAP activity were found in postmenopausal osteoporotic group compared to control group.This result could mean that in bone of normal BMD, there are less active osteoblasts necessary to achieve sufficient fracture healing. More BAP is needed in bone of low BMD and osteoblasts are more activated to balance the remodeling processes in osteoporotic bone. BAP is increased by 77% in women within 10 years of menopause[41].Increased BAP levels are associated with an increased risk of rapid bone loss in pre and postmenopausal women[42].In the present study there was no correlation between BAP and BMD. This is the same finding of the previous studies[43,44].

Conclusions

This study concluded that BAP is limited in diagnosis of osteoporosis because of low sensitivity and specificity. Measurement of OPN levels may be of value in the diagnosis of osteoporosis in postmenopausal women and healthy postmenopausal women, howeverthe measurement of lumbar and hip BMD by DXA is still a gold standardin diagnosis of osteoporosis.

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