



## Evaluation of Osteopontin in Combination with Bone Turnover Markers for the Assessment of Osteoporosis in Postmenopausal Women

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors SR, AS and UR contributed equally in designing the study, writing the protocol, analysis of the samples and writing the first draft of the manuscript. Authors SR, TMA and MM managed the literature searches, data collection and statistical analysis. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/BJMMR/2016/24738

#### Editor(s):

(1) Panagiotis Korovessis, Chief Orthopaedic Surgeon, Orthopaedic Department, General Hospital "Agios Andreas" Patras, Greece.

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(3) Mohamed Ahmed Mahmoud Eid, Cairo University, Cairo, Egypt.

Complete Peer review History: <http://sciencedomain.org/review-history/13714>

Original Research Article

Received 30<sup>th</sup> January 2016

Accepted 7<sup>th</sup> March 2016

Published 16<sup>th</sup> March 2016

### ABSTRACT

**Aims:** Osteoporosis, a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, has an important impact on the lives of postmenopausal women, owing to the increased risk of fractures. Although bone mineral density (BMD) is the standard criteria used for the diagnosis of osteoporosis, but BMD provides a slow and static picture of skeleton whereas, the biochemical markers of bone turnover (BTM) can provide dynamic status of bone remodeling and rapid measurement of skeletal metabolism. Osteopontin (OPN), a

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glycoprotein has been implicated in bone remodeling by activating the resorption process. Combination of osteopontin with classical bone turnover markers can enhance the confidence of detecting osteoporosis and predicting fracture risk.

**Study Design:** Cross-sectional study.

**Place and Duration of Study:** Department of Pathology, Quaid-e-Azam Medical College, Pakistan from 1<sup>st</sup> July 2015 to 15<sup>th</sup> September 2015.

**Methodology:** We included 120 females (60 postmenopausal, age >45 years and 60 from childbearing age 25-45 years) and excluded all conditions affecting bone metabolism. Enzyme-linked immunosorbent assay technique was used to measure levels of bone markers in serum.

**Results:** Bone markers were significantly higher in postmenopausal group of patients. Osteopontin was found to be positively correlated with osteocalcin ( $r=0.82$ ), bALP ( $r=0.76$ ), CTX ( $r=0.62$ ) and DPD ( $r=0.49$ ) and it was negatively correlated with BMD lumbar spine ( $r= -0.71$ ) indicating a significant correlation ( $p<0.0001$ ). The osteopontin and osteocalcin combination showed highest sensitivity (94%) and specificity (88%), closely followed by that of osteopontin and bone alkaline phosphatase combination.

**Conclusion:** High levels of osteopontin in postmenopausal women are associated with low BMD, raised levels of bone turnover markers and fractures. When used in combination with other bone turnover markers, it can provide an accurate assessment of osteoporosis and fracture risk.

*Keywords: Osteoporosis; osteopontin; bone turnover marker; fracture risk; bone mineral density.*

## ABBREVIATIONS

BMD	: Bone Mineral Density
BTM	: Bone Turnover Markers
OPN	: Osteopontin
OC	: Osteocalcin
CTX	: Collagen type 1 cross linked telopeptide / Carboxy-terminal collagen crosslinks
DPD	: Deoxypyridinoline
bALP	: Bone Alkaline Phosphatase
WHO	: World Health Organization
SD	: Standard Deviation
DEXA	: Dual Energy x-ray Absorptiometry
ELISA	: Enzyme-linked Immunosorbent Assay
ROC	: Receiver Operator Characteristics
SPSS	: Statistical Package of Social Sciences
BMI	: Body Mass Index
AUC	: Area Under Curve

## 1. INTRODUCTION

Osteoporosis is a major health problem worldwide. It is defined as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk [1]. It has a tremendous impact on the lives of many postmenopausal women which is expected to increase further due to rise in the aging population [2]. Increased risk for its potentially devastating sequelae of fractures may be the worst consequence, but dependency and poor quality of life of patients living with this disease might be the greatest burden of osteoporosis [3].

It is The World Health Organisation (WHO) diagnostic criterion for osteoporosis is a bone mineral density (BMD) measurement equal to or more than 2.5 standard deviations (SDs) below the young female (age 20–29 years) reference mean (T-score  $F-2.5$  SD) [4-6].

Globally osteoporosis is found more in whites and Asians. Every year approximately 1.5 million fractures are related to osteoporosis. It is predicted that osteoporosis related fracture occur in one out of every two women over 50 years of age [7]. However, in Asia it is estimated that by the end of year 2050, more than about 50% of all hip fractures will be due to osteoporosis [8].

Pakistan is a third world country and there is a steady rise in proportion of the elderly population of the country. It is estimated that Pakistani population will reach 226 million till 2020, out of which approximately 16 million people (7.1%) will be over 60 years of age [7]. This longer life span along with low physical activity, diet deficient in calcium and vitamin D, early menopause, prolonged immobility and illiteracy makes postmenopausal women prone to the risk of developing osteoporosis [9]. According to a survey by International osteoporosis foundation on Pakistani population (2009), it was found that 10 million people ranging between the ages 45-70 years were osteoporotic. The overall prevalence of osteoporosis was found to be 16% [10].

Bone is a specialized form of metabolically active, mineralized connective tissue. It is a

“dynamic tissue” that is remodeled constantly throughout life [11]. In adults bone resorption by osteoclasts is closely coupled with bone formation by osteoblasts to maintain a state of equilibrium until around age 30, after which bone density starts to decline slowly. At the menopause, bone loss is accelerated as estrogen deficiency increases the activity of osteoclasts. Consequently, the net rate of bone resorption exceeds the rate of bone formation, resulting in too little bone, or osteoporosis [12]. The pathogenesis of postmenopausal osteoporosis involves many factors such as aging, hormonal, nutritional, environmental, genetic, and life style factors [13].

Osteoporosis is usually assessed by bone mineral density (BMD) as a measure of bone mass and a predictor of fracture, since bone mass affects bone strength or the ability of a bone to withstand trauma [14]. However in addition to BMD, bone strength and susceptibility to fracture depends on various other factors such as trabecular connectivity and arrangement, biomechanical properties (such as elasticity, strain/stress response, and failure point), bone size, shape, architecture and most important bone turnover. So BMD by itself is not a good detector of osteoporosis and predictor of fracture risk [15,16]. Moreover, BMD changes take almost 18 months to become significant so it provides a slow and static picture of skeleton [17].

We require a more rapid, accurate and dynamic indicator of osteoporosis, also useful in predicting fractures risk. For this purpose, the use of bone turnover markers (BTM) has developed considerably in the past decade [18]. Biochemical markers of bone turnover measured in plasma or urine are proteins or products derived from them. During this process, compounds are released either from bone or from the cells involved in the bone remodeling process (osteoblasts and osteoclasts) [19]. Biochemical markers of bone turnover are broadly divided into two categories:

1. Markers of bone formation, which reflect osteoblast activity
  - Serum total alkaline phosphatase
  - Serum bone-specific alkaline phosphatase
  - Serum osteocalcin
  - Serum type 1 procollagen (C-terminal/N-terminal)

2. Markers of bone resorption, which reflect osteoclast activity

- Urinary hydroxyproline
- Urinary total pyridinoline
- Urinary free deoxypyridinoline
- Urinary collagen type 1 cross-linked N-telopeptide
- Urinary or serum collagen type 1 cross-linked C-telopeptide
- Bone sialoprotein
- Tartrate-resistant acid phosphatase 5b [19-21]

In the course of searching for a better and more reliable marker for osteoporosis, osteopontin was examined in this study. OPN is abundantly distributed in bone and is estimated to comprise approximately 2% of non-collagenous proteins in bone tissue [22] and the name “osteopontin” was proposed to denote that it is a product of bone cells and that it can form bridge (“pons” is Latin for bridge) between cells and the mineral matrix [23]. However, the protein has also been shown to be important in various processes such as angiogenesis, wound healing, inflammatory and immune response [24-26].

OPN is a negatively-charged acidic hydrophilic phosphoglycoprotein which is composed of 300 amino acids and contains an arginine-glycine-aspartic acid cell binding sequence. It is located on the long arm of chromosome 4 region 13 (4q13) and detectable in all body fluids [23]. *Osteopontin* mediates biological functions through signal transduction by binding to the cell surface *receptors* integrin  $\alpha v \beta 3$  and *CD44* [26, 27]. It regulates bone turnover through these receptors. OPN is secreted by both bone cells: osteoblasts and osteoclasts. Osteoclasts are known to highly express  $\alpha v \beta 3$  integrin and *CD44* [28]. OPN not only stimulates migration and adhesion of osteoclasts to bone matrix through the interaction with  $\alpha v \beta 3$  and *CD44*, also increases *CD44* expression on osteoclasts, consequently, initiates a resorptive process as *CD44* expression is necessary for osteoclastic activity [29].

OPN can be used as a promising diagnostic and prognostic indicator of osteoporosis progression in future. But unfortunately there is only limited information available especially about the combination of OPN with other bone turnover markers. Various benefits could conceivably be derived from such combinations. Markers that correlate with OPN and also measures

osteoporosis progression could enhance confidence of early detection and predicting fracture risk. This earlier diagnosis and prevention of fractures will decrease the medical, social and economic burdens of osteoporosis. Yet no algorithms have been developed for such candidate combinations that will facilitate clinical decisions. Tightly controlled large scale studies are required for the full potential of OPN in diagnostic multiplex panels for osteopontin to come to fruition.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

This was a cross-sectional study, carried out in Department of Pathology, Quaid-e-Azam Medical College, Pakistan from 1<sup>st</sup> July 2015 to 15<sup>th</sup> September 2015. The study protocol was approved by ethics committee at Bahawal Victoria Hospital, Pakistan. Written informed consent for participation was obtained from each subject before enrollment.

### 2.2 Study Population

60 women over the age of 45 without menstruation were enrolled into a menopausal group.

#### 2.2.1 Inclusion criteria

- Menopause had occurred at least 2 years before their visit.
- No history of diseases (fractures, etc.) that interfered with the activities of normal daily life.
- No history of osteoporotic fractures
- Had not received radiation therapy or chemotherapy

#### 2.2.2 Exclusion criteria

- Subjects with hormonal replacement therapy or medication affecting bone metabolism (corticosteroids, anticonvulsants, oral anticoagulants, or therapy for osteoporosis).
- Subjects with any condition that might interfere with bone metabolism (thyroid disorders, malabsorption, chronic renal and liver diseases, or alcoholism) were excluded.

Another 60 women, from the ages of 25 to 45 with regular menstruation were enrolled in the control group. The patients were selected in such a way that the groups were comparable in age and sex distribution. Apart from these criteria, selection was random.

### 2.3 Clinical Assessments

Data collected from patients included age, weight, height, body mass index (BMI: weight/height [kg/m<sup>2</sup>]), waist circumference, menarche, and menopause. We also recorded the patients' medication history and associated conditions including hypertension and diabetes.

### 2.4 Sample Collection

After noting the name, age and sex 6 ml of venous samples were drawn into an evacuated tube with aseptic precautions in the morning between 7:00 and 9:00 a.m. after the subject had fasted for 12 hours. Serum was separated by centrifugation at 3000 rpm for 15 minutes at 4° C within 2 hours after venipuncture. To avoid repetitive freeze and thaw cycles, different aliquots of one sample were generated, immediately frozen and stored at -80° C until further analysis.

### 2.5 Sample Analysis

The serum ionized calcium was determined by ion selective electrode (Mindray BS 400). Enzyme-linked immunosorbent assay (ELISA) technique (Elisa reader, Thermo electron corporation MULTISKAN EX) was used to measure serum levels of osteopontin (human osteopontin (OPN); Elabscience, Beijing, Catalog No: E-EL-H1374, sensitivity 0.20 ng/mL; CV <10%), serum collagen type 1 cross-linked C-telopeptide (Human CTX-1; Elabscience, Beijing, Catalog No: E-EL-H0835, sensitivity 0.10 ng/mL; CV <10%), osteocalcin (Human OC; Elabscience, Beijing, Catalog No: E-EL-H1343, sensitivity 0.80 ng/mL; CV <10%), and bone alkaline phosphatase (Human BALP; Elabscience, Beijing, Catalog No: E-EL-H0584, sensitivity 0.46 ng/mL; CV <10%), and urinary deoxypyridinoline (Human DPD; Elabscience, Beijing, Catalog No: E-EL-H1343, sensitivity 4.69 nmol/L; CV <10%). While, vitamin D and estrogen was determined by chemiluminescence (Abbott, architect i1000 SR).

## 2.6 Measurement of BMD

Bone mineral density was measured at the lumbar spine (L2-L4) and at the femoral neck by dual-energy x-ray absorptiometry (DEXA), with Hologic QDR-4500 (Bedford, MA, USA) [CV 1.2% at lumbar spine] at Bahawal Institute of Nuclear medicine and Oncology (BINO), Pakistan. Results were expressed as a T-score (standard deviation from peak adult BMD). Women were classified as osteoporotic (>2.5 SD below the mean value for young adults (according to the World Health Organization's criteria for diagnosing osteoporosis). All the measurements were performed by 2 operators.

## 2.7 Statistical Analysis

All data collected was subjected to standard statistical analysis, such as mean and standard deviation for each of the parameters and expressed as mean±SD. The 95% confidence intervals for the means were calculated. Comparison between postmenopausal and child

bearing age groups for all parameters was done by Student's t-test. Fisher exact test was used for categorical data. The standard linear regression techniques are used to measure Pearson correlation coefficient between quantitative variables according to their distribution. The diagnostic performance of the tests was assessed by using receiver operating characteristic (ROC) curves. All p values were calculated from two-tailed statistical tests, and the statistical significance was assigned to a  $p \leq 0.05$ . The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 17.00, and the graphs in GraphPad prism 5.0 program.

## 3. RESULTS

The main demographic characteristics and other study variables of cases and controls are summarized in Table 1. Increasing concentration of bone markers was associated with age, menopause, estrogen, BMI and bone mineral density.

**Table 1. Characteristics of postmenopausal and childbearing group in the study**

Parameters	Postmenopausal group >45 years	Childbearing group 25-45 years	p-value
Number (N)	60	60	
Age (years)	54.32±4.57	33.83±5.56	<0.0001
Menarche (years)	13.37±1.09	13.08±1.14	<0.0001
Menopause (years)	47.75±1.41	---	
Height (cm)	150.70±5.31	153.52±4.72	>0.05
Weight (kg)	63.74±12.66	61.09±10.84	>0.05
Body mass index (kg/m <sup>2</sup> )	28.65±5.23	27.16±5.12	>0.05
Estradiol (pg/ml)	47.56±36.13	84.32±64.12	<0.0001
(pmol/L)	(174.59±132.63)	(309.53±235.34)	
Vitamin D (ng/mL)	6.74±1.13	9.82±0.98	<0.0001
(nmol/L)	(16.83±2.81)	(24.51±2.46)	
Calcium (mg/dl)	7.16±0.95	9.26±0.69	<0.0001
(mmol/L)	(1.79±0.24)	(2.32±0.17)	
Osteopontin (ng/ml)	16.34±1.51	11.19±1.52	<0.0001
Osteocalcin (ng/ml)	15.32±2.56	9.92±1.59	<0.0001
Carboxy-terminal collagen crosslinks (ng/ml)	2.50±1.78	0.42±0.17	<0.0001
Deoxypyridinoline (nmol/L)	44.83±23.67	19.19±5.57	<0.0001
Bone alkaline phosphatase (ng/ml)	22.36±3.59	14.49±2.55	<0.0001
Lumbar bone mineral density (g/cm <sup>2</sup> )	0.91±0.81	1.22±1.14	<0.0001
Hip bone mineral density (g/cm <sup>2</sup> )	0.77±0.22	0.92±0.13	<0.0001
T-score	-1.57±1.60	0.16±0.78	<0.0001
Z-score	-0.74±1.12	0.28±0.81	<0.0001

### 3.1 Elevated Concentration of Bone Turnover Markers in Postmenopausal Women

Both groups showed a high concentration of both bone resorption and bone formation markers but a significant increase was observed in bone turnover markers in postmenopausal group especially in bone resorption markers indicating that although bone turnover increases after menopause but osteoclastic activity exceeds osteoblastic activity (Fig. 1).

### 3.2 Correlation of Osteopontin with Other Bone Markers

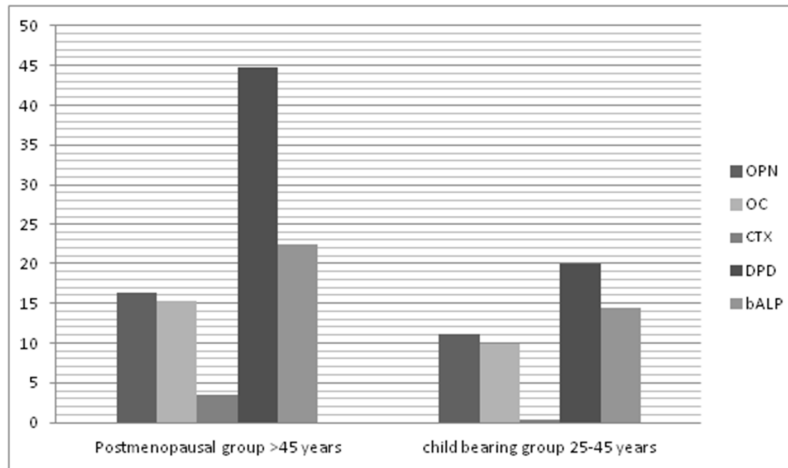
Univariate analysis showed statistically significant correlations between OPN and all other bone turnover markers ( $r=0.49-0.82$ , all  $p < 0.0001$ ), as depicted in Fig. 2.

OPN was found to be negatively correlated with BMD at lumbar spine ( $r = -0.71$ ) and hip bone ( $r = -0.52$ ) while no significant correlation was

observed between OPN and vitamin D (Table 2). Furthermore, Serum OPN levels were correlated with age, body weight, height, T-scores, and Z-scores in post menopausal group but not in childbearing age group.

### 3.3 Sensitivity and Specificity Analysis

ROC analysis was used to assess the diagnostic value of OPN and other bone marker in evaluation of osteoporosis (Table 3). OPN and bone turnover markers were effective for detection of osteoporosis in postmenopausal women with largest area under ROC curve (AUC) observed in OPN 0.91, followed by OC, bALP, CTX, DPD, 0.89, 0.87, 0.76, 0.72 respectively (all  $p < 0.0001$ ) at 95% confidence interval. At the cutoff level of 95% specificity, OPN and bALP showed highest sensitivity. Similar to that, specificity of OPN and bone markers was examined at the cutoff level set at 95% sensitivity. However, at that point specificity of OPN outperformed that of all other bone markers.



**Fig. 1. Serum levels of osteopontin and other bone turnover markers were different between menopausal and childbearing age groups**

OPN: Osteopontin, OC: Osteocalcin, CTX: Carboxy terminal collagen crosslinks, DPD: Deoxypyridinoline, bALP: Bone alkaline phosphatase

**Table 2. Pearson correlation between OPN and other parameters for all subjects**

	Pearson correlation	p-value
Osteocalcin	0.82	<0.0001
Carboxy-terminal collagen crosslinks	0.62	<0.0001
Deoxypyridinoline	0.49	<0.0001
Bone alkaline phosphates	0.76	<0.0001
Bone mineral density (lumbar spine)	-0.71	<0.0001
Bone mineral density (Hip bone)	-0.52	<0.0001
Vitamin D	0.08	<0.0001

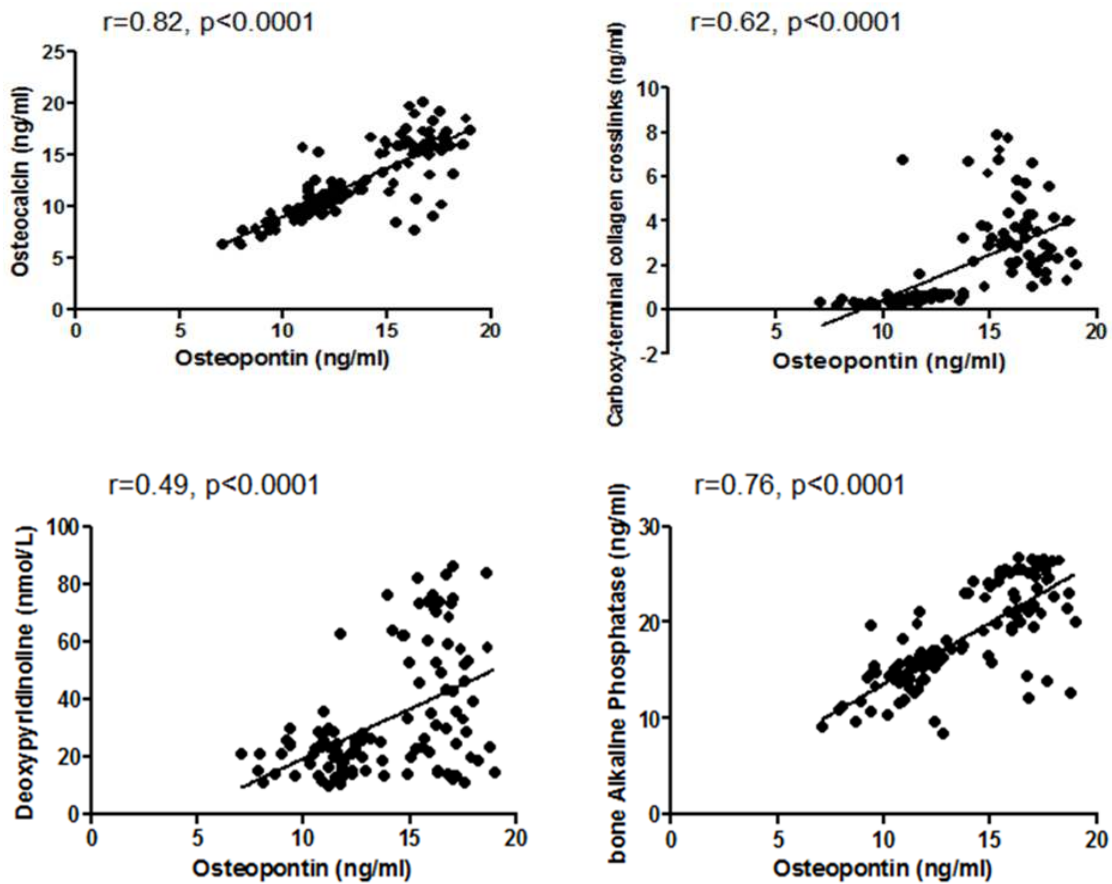


Fig. 2. Correlation of serum osteopontin levels and other bone turnover markers in all study subjects (N=120)

Table 3. Diagnostic sensitivity and specificity and area under curve (AUC) of Osteopontin and other bone turnover markers

Variable	Sensitivity %	Specificity %	AUC
Osteopontin	90	88	0.92
Osteocalcin	86	91	0.89
Carboxy-terminal collagen crosslinks	94	75	0.76
Deoxypyridinoline	88	49	0.72
Bone alkaline phosphates	90	85	0.87

Sensitivity, specificity and area under curve (AUC) with 95% confidence intervals of various markers were calculated using the cutoff level with the highest diagnostic accuracy obtained from ROC analysis.

### 3.4 Analysis of Combination of Osteopontin with Other Bone Markers

The possibility of increasing the diagnostic accuracy in the detection of osteoporosis was examined by means of combination of markers. For this purpose the ROC approach was applied

to identify the significant indicators of bone turnover in osteoporotic patients. This two marker combination resulted in an increase in AUC to 0.94 for OPN and OC, closely followed by 0.91 for OPN and bALP, 0.80 for OPN & CTX while the AUC for OPN & DPD was 0.77 (all  $p < 0.0001$ ) at 95% confidence interval. The highest sensitivity (94%) and specificity (88%) was observed for osteopontin and osteocalcin combination. The osteopontin and bone alkaline phosphatase also showed an excellent sensitivity (92%) and specificity (83%), indicating that if osteocalcin analysis is not available bone

alkaline phosphatase can also be used to evaluate osteoporotic status (Table 4).

**Table 4. The possibility of increasing the accuracy in the detection of osteoporosis and fracture risk was examined by means of combination of osteopontin with other bone markers**

Variable	Sensitivity %	Specificity %	AUC
OPN & OC	94	88	0.94
OPN & CTX	90	76	0.80
OPN & DPD	87	60	0.77
OPN & bALP	92	83	0.91

*OPN: Osteopontin, OC: Osteocalcin, CTX: Carboxy-terminal collagen crosslinks, DPD: Deoxypyridinoline, bALP: Bone alkaline phosphatase, AUC: Area under curve at 95% confidence interval*

#### 4. DISCUSSION

The bone tissue suffers a constant remodeling. But the process of bone resorption by osteoclasts is occurring more rapidly in postmenopausal women as compared to bone formation by osteoblasts. Estrogen contributes to this bone turnover. Postmenopausal women in our study had serum estradiol level below 50 pg/ml with low BMD 0.91 g/cm<sup>2</sup> at lumbar spine, which was less than the control group. Similar results were obtained by Gurban et al. [30,31], who demonstrated the important role of the postmenopausal endogenous circulating estradiol concentrations in the bone turnover process.

Low BMD is related to increased risk of developing osteoporosis and fractures [14,15]. Our results correlated with previous data on BMD and revealed a low BMD, valid at both lumbar spine and hip bone, in postmenopausal women which made them more prone to the development of osteoporosis.

Alone measurement of BMD cannot assess all risk factors for fractures and account for the magnitude of the risk reduction. It will only provide a static picture [21]. So we suggested to evaluate the dynamic process of bone remodeling by measurement of biochemical bone turnover markers, keeping in mind that bone turnover is associated with accelerated bone loss. In this study we showed that level of osteopontin and other bone turnover markers such as osteocalcin, carboxy-terminal collagen crosslinks, deoxypyridinoline and bone alkaline phosphatase is elevated in postmenopausal

group of patients indicating high bone turnover, a crucial step in bone remodeling. Our results are in accordance with Seibel [20] and Hari Kumar et al. [32], who established the use of bone turnover markers as helpful tools to detect the dynamics of the metabolic imbalance. Similar results were obtained by Bahlous et al. [33] in their study which concluded that BTMs provide improved exploration of bone turnover. Garnero et al. [34,35], on the other hand gave contradictory views in their study. According to them due to preanalytical variability and absence of guidelines, the BTMs have poor diagnostic value for assessing osteoporotic status.

A relatively more rise in the level of bone resorption markers i.e. osteopontin, deoxypyridinoline and carboxy-terminal collagen crosslinks is observed in postmenopausal group of patients. This shows that the process of bone resorption by the activity of osteoclasts is the major component of skeletal metabolism in this age group. The results are similar to that of Singer and Eyre [19] who stated that markers of bone formation are somewhat less likely to be elevated than markers of bone resorption. Also according to Vasikaran et al. [17] bone resorption seemed to be stronger predictors of future bone loss than markers of bone formation.

Osteopontin was evaluated in our study as the main bone turnover marker. It reflects the bone resorption process by osteoclasts [36]. The serum level of OPN was markedly elevated in the older age group. Initially various studies on mice were dedicated to study the role of osteopontin and osteoporosis. The previous reports on mice model showed similar results and stated that OPN-deficient mice are resistant to ovariectomy-induced osteoporosis [37]. Our results are comparable with the human studies which discussed the elevated levels of osteopontin as a risk factor for osteoporosis in postmenopausal women [38,39].

Osteocalcin and carboxy-terminal collagen crosslinks (CTX) showed highest correlation with osteopontin, closely followed by bone alkaline phosphatase. They are considered to be efficient markers for evaluation of osteoporotic status in several previous studies [31,35,40].

We have observed that a single bone marker measurement may not fully reflect the ongoing process of remodeling. For example, a bone resorption marker will not provide any information regarding bone formation. Similarly, elevated



bone formation marker may mask the effect of resorption hence, providing a false picture. That is why according to Singer and Eyre [19], the high levels of markers of bone resorption can complement low bone density (BMD) for better diagnosis of osteoporosis and prediction of fracture risk. But our study suggested that combination of osteopontin; a marker for bone resorption, with osteocalcin, a major bone formation marker provides a valuable tool for rapid detection of osteoporosis and can independently predict the fracture risk. The sensitivity and specificity for bone specific isomer of alkaline phosphates in combination with osteopontin were also high indicating that a combination of osteopontin with a single bone formation marker can be used as a better indicator of osteoporotic status. The same conclusion was drawn by Vasikaran S et al. [41] and was published IOF/IFCC (International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine) recommendations in 2011 which suggested the use of one bone formation marker and one bone resorption marker to be used as reference markers and measured by standardized assays in observational and intervention studies in order to enlarge the international experience of the application of markers to clinical medicine and to help resolve uncertainties over their clinical use.

Our study has several limitations. First, the cross-sectional nature of the study implies that no causal inferences can be drawn. The study group was selected from patients who had been referred for DEXA measurement and not from the community, thus explaining the relatively high incidence of osteoporosis. Secondly, there is considerable variability in the biomarkers and lack of adequate standardization of the assays. Residual confounding by factors that we failed to control could have also influenced our findings.

## 5. CONCLUSION

To conclude, our findings suggest that high levels of OPN in postmenopausal women are associated with low BMD, increased levels of bone turnover markers and osteoporotic fracture risk. The high bone turnover during osteoporosis can lead to elevated serum concentration of osteopontin and bone markers. So the measurements of osteopontin in combination with other bone turnover marker especially bone formation markers like osteocalcin and bone alkaline phosphatase may provide useful

information regarding the diagnosis of osteoporosis and assessment of fracture risk.

Further large-scale population data and analyses are needed to confirm these findings and to determine the effects of antiresorptive therapy on BTMs in patients with osteoporosis. Also, there is a current need for establishment of more rapid assays and for improvement in technical methods for measurement of these markers.

In the end we hope that combination of osteopontin with other BTMs may be included in osteoporosis guidelines and fracture risk calculation algorithms.

## DISCLAIMER

This manuscript was presented in the conference.

Conference name: "2<sup>nd</sup> International Conference and Exhibition on Pathology".

Conference link is:  
"<http://www.omicsonline.org/2161-0681/2161-0681-S1.010-008.pdf>"

Date August 05-06, 2013, Embassy Suites Las Vegas, NV, USA.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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