

## ASSOCIATION OF SERUM P1NP (N-TERMINAL PROPEPTIDE OF TYPE1 COLLAGEN) AND BONE MINERAL DENSITY (BMD) IN OSTEOPOROSIS

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### ABSTRACT

**Objective:** To access the variation of P1NP levels in normal, osteopenic and osteoporotic females and to study its correlation of with BMD in pre and post-menopausal women at hip and spine.

**Study Design:** Cross sectional study.

**Place and Duration of Study:** Ziauddin Hospital, Clifton Campus, Karachi during Jan to Dec 2017.

**Material and Methods:** A sample of 267 randomly selected females that fulfilled the exclusion and inclusion criteria were included in the study. BMD was assessed by DEXA Scan while P1NP levels were measured using electrochemiluminescence technique using Roche Cobas analyzer at Ziauddin hospital Karachi. Elecsys 2010, Modular analytics E170 Cobas e immunoassay analyzer. Data was collected using a self-designed Questionnaire. ANOVA was applied to compare mean differences of P1NP levels in pre and post-menopausal women in normal, osteopenic and osteoporotic groups. Regression analysis was used to determine association between P1NP level and BMD levels at spine and hip.

**Results:** A significant negative correlation was found between P1NP and BMD at both the sites, while a strong association was established in P1NP and BMD spine, while for hip bone this association was non significant.

**Conclusions:** Our study concludes that P1NP is a reliable biomarker for prediction of bone density of spine and is negatively correlated with the density.

**Keywords:** Bone mineral density, Osteoporosis, Osteopenia, P1NP.

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### INTRODUCTION

In Osteoporosis decline in bone mass and structural weakening of bone occurs<sup>1,2</sup>. It is a silently succeeding metabolic bone condition that is extensively common in women<sup>2,3</sup>. Osteoporotic fractures are foremost healthcare concern<sup>1</sup>. It can lead to grave disabilities, compromising quality of life<sup>4</sup>. In Pakistan, every year about 8.9 million fractures are resulted from osteoporosis or in added words an osteoporotic fracture occurs after every 3 seconds. Around 9.9 million people in Pakistan are affected by osteoporosis out of which 7.2 million are women. This prevalence is expectedly increase to 11.3 million by 2020 and 12.9 million by 2050<sup>5</sup>. Conversely Pakistan is a developing country and ill-equipped to grasp the burden of osteoporosis<sup>3</sup>.

Bone being metabolically active constantly

undergoes remodeling in a person's life span<sup>2</sup>. It is a highly coordinated process<sup>2</sup>. Osteoclasts are multinucleated bone resorbing cells. Osteoblasts are bone formation cells<sup>6</sup>. Bone Mineral Density (BMD) measurement, predicts bone deterioration or loss. For BMD testing The World Health Organization (WHO) recommends Dual Energy X-Ray Absorptiometry (DEXA) as the gold standard<sup>7</sup> but changes in BMD appear late and are comparatively unalterable at this point<sup>8</sup>. As compare to DEXA scan bone turn over biomarkers unmask early changes in bone metabolism and can be repeated at small interval. Bone Turnover Markers (BTMs) unfold precious Informations for the diagnosis and prognosis of metabolic bone conditions because as compare to DEXA they provide more representative index of overall skeletal bone loss<sup>9</sup>. Thus they are helpful in patient's compliance and reduces cost and extent of treatment. Furthermore portrayal of BMD measurement as an epidemiological screening tool is not logical due to its high cost,

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poor sensitivity to discriminate future fractures and lack of accessibility at every medical center<sup>10</sup>.

Biomarkers of bone formation or resorption are actually cellular components of bone matrix<sup>2,11</sup>. They can be detected in serum and urine<sup>11</sup>. The older biomarkers such as serum calcium, serum alkaline phosphatase and urinary hydroxyproline, due to their poor sensitivity and specificity have been questioned for reliability to assess BMD and ability to monitor treatment response.

The most frequently used bone formation biomarkers which can be detected in serum or plasma include: Bone specific alkaline phosphatase (BSAP), Osteocalcin and the Carboxy- and amino-terminal propeptides of type 1 collagen (P1CP, P1NP)<sup>12</sup>.

Alkaline phosphatase (ALP) was the initial bone biomarker to evaluate bone turnover. Elevated ALP is principally caused by high bone turnover<sup>13</sup> however in several people it had been erroneously taken as normal. In spite of its more specificity, there about 20% chances of cross reactivity with the liver isoform in some diseased states<sup>13-15</sup>.

P1NP has many purposeful leverages and is recommended by the Bone Marker Standards Working Group<sup>14,16</sup>.

Osteocalcin (OC) made by osteoblasts throughout bone formation is the most copious non-collagenous matrix protein in bone<sup>12,17-19</sup>. Osteocalcin is more tissue specific, is wide accessible, and having comparatively low within-person variation<sup>2</sup>. Moreover it is a cost effective test<sup>2</sup>. Its levels are predisposed by renal function, vitamin K status and circadian variation<sup>20,21</sup>. The utility of osteocalcin is restricted by lack of standardization and therefore timely and special handling of the specimen is necessary owing to its instability<sup>22</sup>.

Serum P1NP springs up as a promising bone formation biomarker to review osteoporosis<sup>23,24</sup>. Serum P1NP monitoring is also notably clinically of use as this marker is comparatively not

sensitive to meals intake and circadian rhythm variability. Moreover at normal room temperature it is reasonably stable<sup>22-27,28-31</sup>. The International osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry (IFCC) have recently revealed a comprehensive review on BTMs and suggest PINP as the reference bone forming biomarker<sup>12</sup>. Similarly, the National Bone Health Alliance of United states of America has additionally recommended PINP as the reference bone formation biomarker<sup>12</sup>.

In the present study we plan to evaluate the P1NP as predictors of BMD, for the detection of osteoporosis in Pakistani women.

## **MATERIAL AND METHODS**

A sample of 267 (calculated using survey systems) females of age 35 and above were recruited in the study. Males, pregnant women, past medical history of malignancies, or having signs and symptoms of cholestatic liver disease or with history of jaundice during previous 3 months and females with history of chronic renal failure were excluded.

The study was conducted after approval from Ethics Review Committee of Ziauddin University.

Self-designed structured Questionnaire was used to collect patient information. It included, age, weight, BMI, socioeconomic status, past medical history, family history of fractures, drug history, and demographic variables.

The Hologic Discovery Wi (S/N 88577) DEXA System was used to measure Bone Mineral Density at lumbar spine L1 to L4 and hip. According to the BMD values the patient were categorized as either normal, Osteopenic or osteoporotic.

For Serum P1NP, 5 ml blood samples was drawn in gel tubes. The samples were centrifuged at 4000 rpm for 5 minutes to obtain serum. Serum samples were analyzed by Roche Cobas analyzer at Ziauddin hospital Karachi. Elecsys 2010, Modular analytics E170 Cobas e immunoassay

analyzer, system was used to measure serum P1NP.

**Statistical Analysis**

Data was analyzed by Statistical Package for Social Sciences (SPSS) version 20. ANOVA was applied to compare mean differences of P1NP levels in pre and post-menopausal women in normal, osteopenic and osteoporotic group. For further intra group association of P1NP with BMD sub-groups, post-hoc Games Howel test was applied. Regression analysis was used to determine association between P1NP level and BMD levels at spine and hip. *p*-value of <0.05 was taken as significant.

**RESULTS**

A total of 267 females were recruited in the

BMD was found to be statically non-significant (*p*=0.959). Whereas that of BMD spine was found to be highly significant with a *p*-value of 0.001. Post-hoc Games Howell test was applied to access intra-group association of the P1NP levels with BMD of spine. The summary of the post-hoc test is presented in the table-I.

Logistic regression analysis was used to determine the correlation of BMD of hip as well as that of spine with P1NP. BMD of both the regions showed a significant negative association with P1NP, as shown in the table-II.

BMD was measured at 2 sites for all participants. The sites were total hip and spine. Mean BMD and t-values are shown in table-III.

P1NP levels were significantly different in pre and post-menopausal women as shown in

**Table-I: Association of the P1NP levels with BMD.**

(I) Osteo Group Spine		Sig.
Normal	Osteoporotic	0.002
Normal	Osteopenic	0.008
Osteoporotic	Osteopenic	0.949

**Table-II: Regression analysis of BMD with P1Np.**

	Spearman Correlation	Sig. (1-tailed)
BMD score spine	-0.458	<0.001
BMD score hip	-0.224	<0.001

study. The mean age of our participants was 56.9 ± 81 years. Mean age of pre-menopausal women were 45.1 ± 2.7 years and postmenopausal 57.8 ± 7.3 years

Our sample was comprised of 259 (97%) married, 2 (0.7%) un-married and 6(2.2%) single females. Amongst these, majority were housewives (247 or 92.5%). Premenopausal females were 39 (14.6%) whereas postmenopausal females were 228 (85.4%).

Median P1NP levels of premenopausal women were 46.05 ng/ml ranging from 22.34-132ng/ml and postmenopausal women were 46.05 with range from 15.41-295 ng/ml.

One way ANOVA was applied to test the association of P1NP and BMD of spine as well as that of hip. Correlation of P1NP with hip

table-IV.

**DISCUSSION**

In our study the P1NP levels in postmenopausal group had lower median which can be attributed to the fact that post-menopausal group had wider dispersion of P1NP levels as compare to premenopausal group.

It was suggested by Renis that premenopausal women were having 44.9ng/ml and post-menopausal women were reported mean level of 53.3, Lower levels of P1NP were found with younger age<sup>32</sup> whereas Nomura reported these values to be 46 ng/ml, which is closer to our postmenopausal women. He reported 39.4ng/ml for premenopausal women<sup>33</sup>.

Significant difference in normal to osteopenic and normal to osteoporotic groups

were found. However, no difference in between osteopenic and osteoporotic group was noticed. So, P1NP levels can differentiate between normal to osteopenic and normal to osteoporotic groups. However, P1NP levels can't differentiate between osteopenic and osteoporotic group. Non-significant association between P1NP and hip BMD was found.

A study by Zhao disclosed that women in osteoporosis group had significantly higher P1NP levels as compared to those in normal group. Whereas our study proposed that there is only significant association between BMD spine demonstrating that in our population P1NP can predict BMD of spine confidently whereas that of hip cannot be predicted by this marker.

women using either the and z-score diagnostic criteria by WHO<sup>36</sup>.

It was observed in our study that with both the bone density gradient at hip as well as spine, the levels of P1NP are negatively correlated with high significance. It suggests that P1NP can be the key determining factor of early spine BMD decrease and it can be a good predictor of bone loss. Our results are consistent with the results of other studies. Association was found with a significant difference in the blood PINP concentration among osteoporosis group<sup>33</sup>, normal bone mass group and osteopenia group<sup>37</sup>.

Zhoa suggested that P1NP levels are negatively correlated with BMD at total hip, spine and neck of the femur in postmenopausal

**Table-III: Distribution of P1NP levels and BMD.**

		P1NP levels (ng/ml)	T score hip	BMD score hip	BMD score spine	T score spine
Median		46.05	-1.00	0.90	0.89	-1.20
Range		279.69	4.70	8.90	4.32	7.90
Quartiles	25	38.91	-1.30	0.81	0.74	-2.80
	50	46.05	-1.00	0.90	0.89	-1.20
	75	52.19	0.54	1.04	1.05	0.60

**Table-IV: Association of P1NP levels with menopausal status.**

	Median	Range	Minimum	Maximum	p-value
Premenopausal women	46.05	100.87	22.34	123.2	<0.001
Postmenopausal women	46.05	279.96	15.41	295.1	

A Chinese study reported a significant association between the blood PINP levels and the BMD in post-menopausal women<sup>34</sup>. Non-significant association of P1NP with BMD at hip, spine and femoral neck was concluded by Kharroubi<sup>35</sup>.

Our study demonstrated that, in elderly women whose osteopenia was diagnosed by lumbar spine DXA, serum levels of PINP was raised when compared with age-matched cohorts with normal BMD. This relationship was independent of age and evident at all three anatomical sites that were analyzed by DXA. Furthermore, studies suggest that serum levels of the PINP was significantly elevated in osteopenic

women<sup>34</sup>. The study conducted by Hu on 2799 subjects also reported the negative correlation of P1NP and BMD at spine, total hip and femur neck levels in postmenopausal women<sup>38</sup>. Other studies conducted in China had the same conclusion. Similarly Renis reported that P1NP is significantly and negatively correlated with BMD at spine, total hip and neck of the femur level<sup>32</sup>. In Bosnia and Herzegovina also a significant negative correlation of P1NP and BMD was reported<sup>39</sup>. However Akram was failed to established BMD correlation with P1NP except at spine level<sup>35</sup>. This negative correlation between P1NP levels and BMD indicates a great importance of P1NP levels in predicting bone loss

as has been suggested in several studies. During menopause estrogen decrease cause progressive increases in bone resorption; this effect, coupled with reduced bone formation leads to low BMD, which may account for such correlations.

Regarding the association of P1NP with BMD levels disparities were found even within the population of same country<sup>40</sup>. Likewise some studies suggest strong association of P1NP and BMD at all sites<sup>32,33,38</sup>, while some suggest that BMD at specific sites can be predicted by P1NP while for others it is not a sensitive marker of BMD<sup>35</sup>. From different geographies and ethnicities, nutritional status, dietary supplements use, difference of osteoporotic proportions, age, weight, smoking habits and physical activities as well as intra individual and inter assay differences, diverse and contradicting results are found. This necessitates further studies in our population to assess the clinical utility of the marker in diagnosis as well as management of BMD related diseases.

## CONCLUSION

Serum P1NP levels were found to be significantly associated with bone mineral density at spine, whereas that of hip was found to be non-significant. There levels were negatively correlated with the BMD at both the sites. Whereas the P1NP levels were found significantly associated with menopausal status.

## CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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