Original Research Article

Role of biochemical markers in the prediction of osteopenia and osteoporosis in men and women of Sikkim, India

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ABSTRACT

Background: Osteoporosis being a silently progressing disease, the real challenge is to identify the individual at high risk of osteoporosis. Many bone turnover marker have been associated with bone loss even before occurrence of any changes in bone structure. Therefore, this study was aim to evaluate the predictive value of bone turnover marker by correlating with low bone density.

Methods: This was a case control study conducted in Sikkim Manipal Institute of Medical Sciences, India. A total of 300 subjects (150 case and 150 control) both male and female between the age group of 35- 65 were enrolled. We measure one bone formation marker serum osteocalcin and two resorption marker urine hydroxyproline (OHP) and urine N- terminal telopeptides of type I collagen (NTX). Calcaneal QUS device (GE Lunar Achilles Express heel-densitometer) was used to determine the bone density.

Results: A significant difference of the bone markers i.e. hydroxyproline, NTx and osteocalcin were observed between cases and control of men and women with $P<0.001$. These variables statistically significantly predicts bone density with $F_{(3, 71)} = 5,671$, $P= 0.002$, $R^2= 0.193$ and $F_{(3, 71)} = 5.292$, $P= 0.002$, $R^2= 0.183$ in women and men respectively.

Conclusions: Study showed that bone turnover markers are able to predict low bone mass. Resorption markers NTx and OHP were the main predictor in men whereas OHP and formation marker Osteocalcin in women. Therefore, BTM measurement along with BMD can provide useful information about the changes in the bone mass which will help to predict the risk of osteoporosis.

Keywords: Bone turnover marker (BTM), Hydroxyproline (OHP), N-terminal telopeptides of type I collagen (NTX), Osteocalcin

INTRODUCTION

Osteoporosis is a disease characterized by low bone mass and micro architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk.[1] It is a silently progressing disease therefore, remains undetected until a fracture occurs on mild trauma. Various combinations of measurements and risk factors have also been tested for predicting osteoporotic fractures. According to a study by Durosier C et al, these fall into three categories.[2] The first category includes absorptiometry (radiographic, single photon and single X-ray, dual photon and dual X-ray), quantitative computed tomography (QCT) and peripheral QCT, quantitative ultrasound (QUS), and magnetic resonance imaging (MRI). Of these techniques, only the latter three-QCT, QUS, and MRI-have substantial potential to provide information about bone quality and

structure beyond BMD. The second category is assessment of clinical risk factors and the third category is bone markers.

According to the studies the use of biochemical markers of bone turnover as indicators of overall bone metabolism has been a potentially valuable clinical method for screening, diagnosis, and monitoring of osteoporosis.[3] Biochemical markers reflect small changes in bone turnover of the entire skeleton in a shorter time frame compared with absorptiometry method, and also capture bone properties independent of BMD measurements.[4,5]

Therefore this study was aim to evaluate the predictive value of bone turnover marker by correlating with low bone density.

METHODS

This was a case control Study conducted in Sikkim Manipal Institute of Medical Sciences, India and the study protocol were approved by Institutional Ethics Committee (IEC no- SMIMS/IEC/2012/PhD-3). The sample size was calculated using difference in means formula with alpha 5 percent.[6]

It was found that a minimum of 232 (116 case and 116 control) subjects needed to be enrolled in the study. Therefore, a total of 300 subjects (150 cases and 150 control) were recruited. Of 150 cases 75 were women and 75 were men and similarly in control group 75 were men and 75 were women. A detail medical history of both men and women and, menstrual history of women was obtained. Informed consent was obtained from all the subjects enrolled in the study.

Subjects with liver disorders, bone tuberculosis, alcoholic liver diseases, hyper/hypothyroidism, chronic renal disease, cancer, hyper/hypo parathyroidism and women on long term medication affecting the bone turnover (steroids, heparin and warfarin and hormone replacement) were strictly excluded. Subjects consuming alcohol and smokers were also excluded. None of the women in the study were pregnant or lactating.

Bone mineral density measurement

The bone mineral density was measured at the calcaneus of right foot by QUS (GE Lunar Achilles Express heel-densitometer) as DEXA was not accessible in this area.

Biochemical measurement

Venous blood sample (5ml) and 24hr urine Sample were collected after overnight fasting. Level of serum calcium, phosphorus, Alkaline phosphatase (ALP) and albumin were estimated by semi auto analyzer (Erba CHEM-5 Plus v2, Mannheim.) by using commercially available kits by Pointe Scientific and Erba Mannheim. The reference range of serum calcium was 8.5-10.4mg/dl. The

intra and inter assay coefficient of variation were 0.9-1.3% and 1.1- 1.3% respectively. The reference range of serum phosphorus was 2.5-4.8mg/dl. The intra and inter assay coefficient of variation were 0.9-1.4% and 2.3-2.7% respectively. The reference range of serum albumin was 3.5- 5 g/dl. The intra and inter assay coefficient of variation were 1.46-1.63% and 2.06-3.23% respectively.

Bone turnover markers

Bone formation

Serum osteocalcin was measured by enzyme linked immunoassay using the Microvue Bone Health osteocalcin kit (Quidel, SanDiego, CA, USA). The sensitivity is 0.45ng/ml, and the intra- and interassay CV were 4.8-10.0% and 4.8-9.8%, respectively.

Bone resorption

Urinary N-terminal telopeptides of type I collagen (NTX) were quantified by Osteomark NTx urine enzyme immunoassay (EIA) kit (Alere Scarborough, ME, USA). The sensitivity is 20 nM BCE, and the intra- and inter assay CV were ≤20% and 7-10%, respectively. Measurement of the urine hydroxyproline was based on a modification of the method described by Neuman and Logan.[7]

Statistical analysis

Data were analyzed using SPSS software version 16. Student's t test was performed to compare the continuous data of cases and control. Relationship between the continuous data was analyzed by Pearson correlation method and association by linear regression analysis for osteocalcin, OHP and NTX with T-score of BMD. The comparison of pre and post-menopausal women with respect to BMD and bone markers was done by T test. $P<0.05$ was considered as statistically significant.

RESULTS

General characteristics of the studied population are presented in Table 1 and 2 for women and men respectively. In women significant difference in T score of BMD and bone markers i.e. hydroxyproline, NTx and osteocalcin were observed between cases and control with P<0.001. Similar result was also observed in men with p<0.001.

Relationship between the bone markers and the other variable in men and women is shown in Table 3. In the women population the resorption maker NTx had significant negative correlation with T score and positive correlation with age (r= -0.691, P= 0.000 and r= 0.416, P= 0.000) respectively. Hydroxyproline also had positive correlation with age and negative correlation with T score (r= 0.291, P= 0.000; and r= -0.663, P= 0.000) respectively besides this, hydroxyproline also had a

positive correlation with ALP and albumin (r= 0.231, P= 0.004 and r= 0.187, P= 0.022) respectively and negative correlation with phosphorus (r=-0.213, P= 0.009).

Formation marker osteocalcin had a significant negative correlation with T score of BMD (r= -0.341, P= 0.000) but no correlation was observed with age.

Table 1: Characteristics of case and control group women.

Characteristics	Control (n= 75) Mean±SD	Cases (n= 75) Mean±SD	P value
Age (year)	44.9±9.4	51±9.9	<0.001
Height (cm)	162.5±6.6	154.5±7.5	0.984
Weight (Kg)	58.9±9.7	58.8±11.5	0.939
BMI (Kg/m2)	24.7±3.8	24.6±4.4	0.84
T-score	-0.19±0.6	-1.98±0.58	<0.001
Serum calcium (mg/dl)	9.8±0.37	9.8±0.43	0.95
Serum ALP(IU)	85.6±26.9	91±31.2	0.265
Serum albumin (mg/dl)	3.9±0.25	4±0.4	0.063
Serum osteocalcin (ng/ml)	8.2±3.2	12.4±4.3	<0.001
Urine OHP	32.3±13.7	55.4±11.9	<0.001
Urine NTX (nmol BCE/ mmol cr)	46±19.1	103.4±24	<0.001

Table 2: Characteristics of case and control group men.

Characteristics	Control (n= 75) Mean±SD	Cases (n= 75) Mean±SD	P value
Age (year)	43.4±7.4	48.4±9.6	<0.001
Height (cm)	165.1±6.7	164.9±7.1	0.809
Weight (Kg)	65.2±10.9	65.2±10.2	0.975
BMI (Kg/m^2)	23.8±3.5	23.8±3.2	0.996
T-Score	.03±0.67	-1.7±0.51	<.001
Serum calcium (mg/dl)	10.2±0.49	10.2±0.5	0.624
Serum phosphorus (mg/dl)	3.7±0.5	3.8±0.6	0.413
Serum ALP (IU)	98.8±38	114.1±29.9	0.007
Serum albumin (mg/dl)	4.0±0.48	4.0±0.5	0.647
Serum osteocalcin (ng/ml)	7.2±2.5	15.3±7.3	<0.001
Urine OHP	28.6±9.9	51.1±12.2	<0.001
Urine NTX (nM BCE/mM Cr)	33.1±2.5	88.6±18.9	<0.001

Table 3: Correlation of bone markers with other variables in men and women.

		Osteocalcin		NTX		Hydroxyproline	
		Men	Women	Men	Women	Men	Women
Age(yr.)	r	0.258**	0.043	0.418**	0.416**	0.255**	0.291**
	p	0.001	0.605	0.000	0.000	0.002	0.000
BMI (Kg/m^2)	r	-0.025	-0.059	-0.068	-0.017	0.048	0.046
	p	0.764	0.475	0.405	0.836	0.563	0.580
Serum Calcium (mg/dl)	r	0.025	0.087	-0.049	-0.033	0.061	0.103
	p	0.759	0.292	0.549	0.690	0.460	0.211
Serum phosphorus (mg/dl)	r	-0.007	-0.148	0.042	-0.040	0.043	-0.213**
	p	0.933	0.072	0.607	0.624	0.599	0.009
Serum ALP (IU)	r	0.123	-0.076	0.230**	0.091	0.130	0.231**
	p	0.134	0.356	0.005	0.268	0.114	0.004
Serum Albumin (mg/dl)	r	-0.120	0.067	-0.070	0.147	0.066	0.187*
	p	0.144	0.412	0.393	0.073	0.421	0.022
T-score	r	0.479**	0.341**	-0.752**	-0.691**	-0.626**	-0.663**
	P	0.000	0.000	0.000	0.000	0.000	0.000

**- Correlation is significant at the 0.01 level; *- Correlation is significant at the 0.05 level, r- Correlation coefficient; P- probability.

Table 4: Linear regression analysis of bone markers and T score in women.

Bone markers	Dependent variable	Case (75)				Control (75)			
		B	SE	P value	95% CI	B	SE	P value	95% CI
OHP	T score	-0.018	0.005	0.001*	-0.029- (0.008)	-0.008	0.005	0.153	-0.018- 0.003
NTx	T score	-0.001	0.003	0.870	-0.006- 0.005	-0.001	0.004	0.789	-0.009- 0.007
Osteocalcin	T score	-0.038	0.015	0.012*	-0.009- 0.067	-0.001	0.022	0.974	-0.043- 0.045
		$R^2 = 0.193$				$R^2 = 0.033$			

*, $p < 0.05$; R^2, Coefficient of determination (% of the variance that the model explains for each variable)

Table 5: Linear regression analysis of bone markers and T score in men.

Bone markers	Dependent variable	Case (75)				Control (75)			
		B	SE	P value	95% CI	B	SE	P value	95% CI
OHP	T score	-0.010	0.005	0.036*	-0.019- 0.000	-0.035	0.008	0.779	-0.019- 0.014
NTx	T score	-0.011	0.003	0.001	-0.017- (-0.004)	0.059	0.006	0.645	-0.010- 0.015
Osteocalcin	T score	-0.015	0.008	0.051*	0.000- 0.030	-0.116	0.032	0.345	-0.094- 0.033
		$R^2 = 0.183$				$R^2 = 0.014$			

*, $p < 0.05$; R^2, Coefficient of determination (% of the variance that the model explains for each variable)

In men population NTx had significant positive correlation with age and ALP (r= 0.418, P= 0.000 and r= 0.230, P= 0.005) respectively and negative correlation with T score (r= -0.752, P= 0.000). Hydroxyproline had significant positive correlation with age (r= 0.225, P= 0.002) and negative correlation with T score (r= -0.626, P= 0.000). Similarly, osteocalcin had significant positive correlation with age (r= 0.258, P= 0.001) and negative correlation with T score (r= -0.479, P= 0.000).

A linear regression was run shown in Table 4 and 5 for women and men respectively to predict bone density from bone markers OHP, NTx and osteocalcin. These variables statistically significantly predicts bone density, F (3.71) = 5,671, P= 0.002, R2= 0.193. Of the three markers OHP and osteocalcin added statistically significantly to the prediction, P<0.05 in women. In men these variables also statistically significantly predicted bone density, F (3, 71) = 5.292, P= 0.002, R2= 0.183 and the resorption markers OHP and NTx added statistically significantly to the prediction, P<0.05.

Table 6: Comparison of T score and BTM between pre and postmenopausal women by t test.

	Premenopausal (N= 81) Mean±SD	Postmenopausal (N= 69) Mean±SD	P value
T score	-0.79±1.08	-1.44±0.96	<0.001
OHP	40.32±18.36	48.10±15.0	0.006
NTx	62.65±37.89	88.95±27.94	<0.001
Osteocalcin	10.35±4.74	10.41±3.88	0.935

As menopause is consider on of the important factor for low bone density, comparison of bone markers and T Score between pre and postmenopausal women by T test

is shown in Table 6. T score was significantly lower among postmenopausal group with P<0.001. NTx and hydroxyproline was significantly higher in postmenopausal with p<0.001 and P= 0.006 respectively but there was no significant difference observed in osteocalcin level.

DISCUSSION

Men in their fifties do not experience the rapid loss of bone mass that women do in the years following menopause. By age 65 or 70, however, men and women are losing bone mass at the same rate, and the absorption of calcium, an essential nutrient for bone health throughout life, decreases in both sexes.

According to studies high rate of bone turnover correlates with low bone mass. Several prospective studies have shown that an increased bone resorption evaluated by specific biochemical markers was associated with increased risk of the hip, spine and non-vertebral fractures independently of BMD.[8-10] Serum osteocalcin a formation marker reflects the 10-40% of osteocalcin produced that is not incorporated in the bone matrix. In postmenopausal women serum osteocalcin level correlates significantly with both the bone formation rate and the kinetically determined calcium accretion rate.[11]

In our study, significant difference (P<0.001) in the bone markers between the case and control of both men and women have been observed. All the bone markers shows a significant positive correlation with the age at P<0.001. T score of the BMD showed a significant negative correlation with all the bone markers.

On grouping the women into pre and postmenopausal subjects the level of resorption markers NTx and OHP

were significantly higher in postmenopausal group but, no significant difference was observed for osteocalcin suggesting that rapid rate of bone resorption could not be compensated by the rate of formation.

On performing linear regression analysis for the association of BMD with low bone density, the bone markers could statistically significantly predict the bone mass. Although only the resorption marker contributed to the prediction in case of men suggesting that in men osteoporosis mainly occurs as a result of increase resorption of bone. In women OHP and osteocalcin contributed for the prediction suggesting the imbalance of formation and resorption of bone metabolism.

CONCLUSION

In conclusion, this study showed that bone turnover marker is significantly associated with bone mass. Resorption markers OHP and NTx turned out to be a strong predictor of low bone mass in men whereas both formation marker osteocalcin and resorption marker OHP in women. Therefore, BTM measurement along with BMD can provide useful information about the changes in the bone mass which will help to predict the risk of osteoporosis.

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