Original Article

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Anatomy Section

Bone Mineral Density: Relationship with Serum Osteoprotegerin, Biophysical Profile and Menopausal Status in Indian Women

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ABSTRACT

Introduction: The Osteoprotegerin (OPG) cytokine network has been found to control bone homeostasis and is implicated in increased skeletal resorption, including several forms of osteoporosis.

Aim: To establish the relationship of Bone Mineral Density (BMD) with serum Osteoprotegerin levels and the biophysical profile (waist to hip ratio and body mass index) and also to correlate it with menopausal status and risk of osteoporosis.

Material and Methods: A case-control study was conducted on 110 non-pregnant subjects: 40 peri-menopausal, 40 post-menopausal women and 30 women in the reproductive age group.

Bone mineral densitometry by Quantitative Computerized Tomography (QCT) at lumbar spine, Waist-to-hip (W:H) ratio and Body Mass Index (BMI) were computed.

Results: Independent student's t-test and Karl Pearson's correlation coefficient analyzed the parameters under study. Standard unimodal distribution curve determined the cut-off value of Osteoprotegerin for screening of osteoporosis risk. In the peri-menopausal age group (40-55 years), there were significant correlations of BMD with age and BMI (p=0.0857; p=0.458). The postmenopausal group (50-70 years) had significant correlations of OPG with age (p=0.030), W:H ratio (p=0.002) and BMI (p=0.005). An OPG concentration of 17.9 pmol/L could predict osteoporosis in postmenopausal women. BMD detected the maximum number of osteoporotic and osteopenic subjects within the postmenopausal group while least were encountered in the normal group.

Conclusion: Osteoporosis was encountered in 60% of post-menopausal and 40% of peri-menopausal patients due to low oestrogen levels. Maximum derangement of BMD, waist to hip ratio and BMI were seen in overweight women. The significant correlations of BMD with BMI may be utilized for assessing osteoporosis in menopausal women.

Keywords: Body mass index, Bone mineral density, Waist-to-hip ratio

INTRODUCTION

Osteoporosis is the most prevalent bone problem in the elderly worldwide characterized by low Bone Mineral Density (BMD) and micro-architectural deterioration, which eventually manifests as low back pain, loss of height over time, a stooped posture or a bone fracture that occurs much more easily than expected.A complex regulatory system of systemic and local factors such as sex hormones, calcium regulating factors, growth factors and cytokines (IL-1, IL-6, IL-11, TGFβ and TNFα) maintain the crucial balance between bone formation and resorption [1]. After middle age, a large segment of population shows a decline in bone growth with ageing which becomes more pronounced in menopausal women than men. Menopause is defined retrospectively when menstruation permanently ceases and the oestrogen deficiency invariably leads to an increased risk of osteoporosis due to the net reduction of post-menopausal bone mass consequential to increased bone remodelling. As adipose tissue serves as a major site of extra glandular oestrogen production, its formation may actually be enhanced in obese post-menopausal women leading to the worsening of cardiovascular risk factors associated with weight gained during the menopausal transition [1-3]. Oestrogen directly modulates the production of osteoclast-stimulating and inhibiting factors and the cells of the osteoblastic and osteoclastic lineage tightly regulate the bone formation and resorption through two key regulators: Osteoprotegerin (OPG) and Receptor Activator of Nuclear factor Kappa B Ligand (RANKL). OPG was initially described in the regulation of bone density [4-6]. This was supported by Mizuno A et al., who observed severe osteoporosis in mice lacking OPG and later Tsuda E et al., described a cytokine network: the tripartite OPG, RANK, RANKL, which was

found to control bone homeostasis [5,7]. This system was implicated in various skeletal and immune mediated diseases including several forms of osteoporosis, rheumatoid arthritis and acute vascular syndromes [8]. Since then, much research has been conducted about their developmental, homeostatic and pathological roles in skeletal and immune biology [8-10].

Due to conflicting reports of association of OPG with the parameters under investigation in the study, this study aimed to establish the inter-relationships of BMD with serum OPG levels, biophysical parameters and menopausal status. The medical implications of this study are not only limited to the menopausal state but also in dealing with various pathological conditions involving calcifications of tissues and bone diseases.

MATERIALS AND METHODS

A case-control study was conducted by the Department of Anatomy in collaboration with the Departments of Obstetrics and Gynaecology and Radiology. From October 2008 to March 2010, a total of 110 non-pregnant subjects were included in the study after obtaining informed consent from all individual participants. The study was in accordance with the ethical standards of the Indian Council for Medical Research.

Patient Selection Criteria:

• **Study Group A:** Forty perimenopausal women (age: 40 to 55 years) with evidence of irregular menstruation (an abnormal variation in length of menstrual cycle ranging from eight to twenty days, between the shortest and the longest cycle lengths) and menopausal symptoms, associated with elevated serum FSH levels (>20 IU/L).

• **Study Group B:** Forty Post-menopausal women with complete cessation of menstruation and serum FSH >20 IU/L and LH>30 IU/L. Only women menopausal for more than five years duration were included in this study.

• **Normal Group:** Thirty healthy women (recruited from patients attending gynaecology OPD) in the reproductive age group were included in the study to set up normal standards in the Indian female population, according to the assay procedure.

Only patients advised CT Scan-Abdomen were recruited, to avoid unnecessary radiation exposure.

Exclusion Criteria:

• Patients of premature, delayed and artificial menopause and women with oligomenorrhea or amenorrhoea before the age of 40 years.

• Patients with any acute coronary syndrome, chronic infections, history of diabetes, tuberculosis, hypertension, renal/cardiovascular/autoimmune disease, bleeding disorder, cancer and epilepsy.

• Patients with history of long term drug intake (especially calcium supplements, corticosteroids, oral contraceptive pills).

During the course of investigations, patients presenting with deranged blood parameters were excluded from this study.

The Written/Informed patient consent with all the details of the history, clinical examination and investigations were recorded and made available to the patient. A detailed clinical history was taken. Any medical history of chronic drug intake and operative procedures underwent, were ruled out. Clinical examination included general physical and systemic examinations. For blood pressure measurement, two readings were taken at an interval of six hours. Korotkoff phase 5 was used to define diastolic blood pressure. BMI was calculated with weight (in kilograms) measured by standardized weighing machine and Height (in metres) by measuring tape.

Body Mass Index (BMI) = Weight (Kg)/Height^2(m) {Acceptable range: 18.5-24.9; Overweight: 25.0-29.9; Obese: 30.0-39.9; Morbidly obese: >40}

Waist-to-hip ratio was calculated using a measuring tape: Waist-hip ratio (cm) = Waist circumference/Hip Circumference (Abnormal >0.9)

Investigations: Complete haemogram; Blood sugar-fasting and post parandial; Lipid profile; Liver function tests; Kidney function tests; Serum FSH and LH.

BMD: QCT for bone density was performed at lumbar spine. BMD is expressed as a T-score, which is the number of standard deviations from the mean for a young healthy woman. T-score of 0 to -1 is considered normal, osteopenia:-1 to -2.5 and a score below -2.5 indicates osteoporosis.

Estimation of Serum Osteoprotegerin levels by ELISA technique: Blood obtained by venepuncture was collected and centrifuged for sera. The assay was done by ELISA technique using MICROVUE OPG enzyme immunoassay kit which provides materials for quantitative determination of total human Osteoprotegerin (OPG) in different experimental sample types. The antibodies used in this test are human OPG specific with no detectable cross-reactivity to soluble form of RANKL and TRAIL (TNF- related apoptosis inducing ligand) at 120 pmol/L.

Calculation of Result: The average optical density was calculated for each of the standards and samples with the help of the Sirio S ELISA reader. A standard curve was created by plotting the optical density for each standard concentration on the ordinate against OPG concentration on the abscissa and a best fit curve was drawn. To determine the concentration of OPG for each sample, first the mean absorbance value was found on the ordinate and then a horizontal line was extended to the standard curve, at the point of intersection, a vertical line was extended to the abscissa and the corresponding OPG concentration was read.

STATISTICAL ANALYSIS

The parameters of the study groups were compared and p-value was calculated using independent student's t-test: p-value < 0.001 - very highly significant; p-value <0.01 - highly significant; p-value <0.05 – significant (p1: denotes p-value for group A versus normal group, p2: p-value for group A versus group B and p3: p-value for group B versus normal group). The correlations between BMI and waist-hip ratio with serum Osteoprotegerin and BMD were calculated using Karl Pearson's correlation coefficient. A standard unimodal distribution curve was used for determining the cut-off value of Osteoprotegerin for screening of patients for osteoporosis risk and all statistical evaluations were performed using SPSS (Statistical Package for Social Science) 16.0 version.

RESULTS

The statistical difference in age between the three groups was very highly significant (p<0.001) while the mean values of body mass index and waist to hip ratio were comparable. In relation to age, mean BMI was found to be higher in normal and perimenopausal than postmenopausal group [Table/Fig-1]. Mean serum Osteoprotegerin levels showed an increase with age and were significantly higher (p=0.013) in the post-menopausal group B [Table/Fig-1].

In 110 subjects, BMD detected 48 (44%) patients with osteoporosis [Table/Fig-2], 37 patients (34%) with osteopenia [Table/Fig-3] and 25 (22%) with normal [Table/Fig-4] bone scans. The postmenopausal group had the maximum number of osteoporotic and osteopenic subjects while least were encountered in the normal group [Table/Fig-5]. In the postmenopausal group, age had significant correlation with bone mineral density and this correlation did not exist within other groups(p1=0.007) [Table/Fig-2].

The clinical and laboratory parameters of the three groups have been tabulated and statistically analysed as follows:

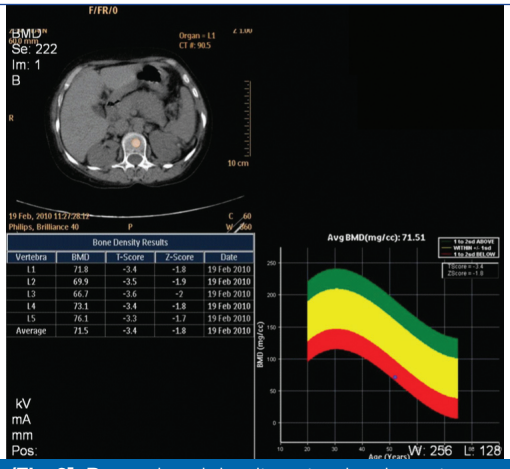
Peri-menopausal Group A: Age range of the 40 subjects was from 40- 55 years [Table/Fig-1]. Age positively correlated with waist to hip ratio and OPG levels but only the correlation of age with BMI was found to be statistically significant [Table/Fig-6]. Body mass index was in the range of 18.18-40.35. Twenty-nine (72.5%) patients with abnormal BMI were stratified accordingly and maximum number of patients with deranged BMD was found in the overweight and obese groups [Table/Fig-1,7]. Waist-hip ratio ranged from 0.76 -1.17. 21 (53%) had W:H ratios greater than 0.9 and were overweight with altered BMD [Table/Fig-8]. Mean serum OPG levels were highest in patients who were found to be osteoporotic [Table/Fig-5]. The correlations of serum OPG with other parameters under study were not found to be significant [Table/Fig-6]. BMD in this group estimated 40% patients to be osteoporotic, 33% osteopenic and 27% with normal BMD with no other significant correlations [Table/Fig-5].

Post-menopausal Group B: The age of the subjects ranged from 50-70 years, with a positive significant correlation of age with BMI and OPG [Table/Fig-6]. BMI was found to be ranging from 17.54-31.6. In 24 patients (60%) with abnormal BMI, the overweight patients showed maximum derangement of waist: hip ratio (67%) and no patient had normal BMD. The OPG levels in subjects with altered BMI were elevated in 20 (83%) patients [Table/Fig-1,7]. BMI positively and significantly correlated with age, W:H ratio and OPG levels [Table/Fig-6].

W:H ratio ranged from 0.68-1.11.In 24 (60%)patients with abnormal W:H ratio, 16 (67%) had increased BMI, out of which the overweight patients with altered BMD predominated [Table/Fig-1,8]. Waist: hip ratio had a positive and significant correlation with BMI and OPG levels [Table/Fig-6]. Mean serum OPG levels were highest in the 66-70 age group and was positively and significantly correlated with age, BMI and W:H [Table/Fig-1,6]. Maximum osteoporotic and osteopenic patients were found within this group with only three patients

Parameters	Normal Group N=30	Group A Perimenopausal N=40	Group B Postmenopausal N=40	p-value
Mean age (Years)	32.30±5.60	46.55±3.52	58.25±6.28	p1=0.000* p2=0.000** p3=0.000***
Mean Body Mass Index (kg/m^2)	26.27±4.33	26.63±4.57	25.20±3.5	p1=0.739* p2=0.120** p3=0.257***
Mean Waist to Hip ratio	0.93±0.97	0.92±0.87	0.92±0.10	p1=0.615* p2=0.799** p3=0.818***
Mean OPG (pmol/L)	10.85±6.57	12.71±9.70	15.36± 7.88	p1=0.368* p2=0.184** p3=0.013***

[Table/Fig-1]: Comparable parameters of Normal, Perimenopausal and Postmenopausal groups.
p1*: Denotes p-value for Normal Group versus group A (Perimenopausal women); p2**: Denotes p-value for Group A (Perimenopausal women) versus Group B (Postmenopausal women); p3***: Denotes p-value for Group B (Postmenopausal women) versus Normal Group



[Table/Fig-2]: Bone mineral densitometry showing osteoporosis by QCT.

[Table/Fig-3]: Bone mineral densitometry showing osteopenia by QCT.

[Table/Fig-4]: Bone mineral densitometry showing normal bone scan by QCT.

with normal BMD. The correlations of BMD with age and BMI were positive, and the relationship was significant (p=0.001; p=0.001) [Table/Fig-5].

Group	Normal BMD (N=25)	Osteopenia (N=37)	Osteoporosis (N=48)
Normal Mean age(years) Mean BMI (Kg/m^2) Mean OPG (pmol/l)	N=11 (37%) 30.90±4.70 26.68±3.58 11.45 (5-27)	N=11(37%) 34.00 ± 4.92 26.01±4.28 10.41 (5-21)	N=8(27%) 31.88 ± 7.50 26.05±5.72 10.62 (5-16)
Perimenopausal Mean age (years) Mean BMI (Kg/m^2) Mean OPG(pmol/l)	N=11(27.5%) 46.09±3.83 28.11±5.36 9.14 (1.5-19.5);	N=13(32.5%) 46.54 ± 2.47 25.94±4.40 7.19 (1.5-18.5)	N=16(40%) 46.88 ± 4.15 26.17±4.18 19.72 (5-38.5)
Postmenopausal Mean age (years) Mean BMI (Kg/m^2) Mean OPG (pmol/l)	N=3(7.5%) 50.67±1.15 19.68± 3.31 10.5 (9.5-11.5)	N=13(33%) 54.85 ± 4.18 24.02±2.89 9.35 (0-16.5)	N=24(60%) 61.04 ± 5.95 26.52±2.97 19.24 (5-34)
Mean Serum OPG (pmol/l)	10.32	8.90	17.95

[Table/Fig-5]: Comparison of various parameters in relation to Bone Mineral Density.

	Normal	Perimenopausal	Postmenopausal
Correlation of age with:			
Body Mass Index	r = -0.052 p = 0.785	r = 0.347 p= 0.028	r = 0.333 p= 0.036
Waist-Hip ratio	r = 0.037 p = 0.844	r = 0.049 p = 0.764	r = 0.237 p = 0.141
Osteoprotegerin concentration	r = -0.097 p = 0.610	r = 0.075 p =0.647	r = 0.343 p =0.030
Correlation of body mass index with:			
Waist-Hip ratio	r = 0.781 p = 0.000	r = 0.234 p = 0.147	r = 0.446 p = 0.004
Osteoprotegerin concentration	r = -0.111 p = 0.559	r = -0.112 p =0.492	r = 0.432 p =0.005
Correlation of waist-hip ratio with:			
Osteoprotegerin concentration	r = 0.119 p = 0.531	r = 0.213 p =0.186	r = 0.484 p =0.002

[Table/Fig-6]: Inter-relationships of various parameters in the three groups

Normal Group: Age of 30 subjects was found to be ranging from 20-40 years. Body mass index ranged from 18.76-35.55 kg/m^2. A total of 21 (70%) subjects with abnormal BMI were overweight and showed maximum derangement of W:H ratio and BMD [Table/Fig-7]. BMI had a positive and very highly significant correlation with W:H ratio (p<0.001) [Table/Fig-6]. Waist-hip ratio was found to be ranging from 0.71-1.10, with 19(63%) patients having abnormal W:H ratios, out of which maximum were overweight with altered BMD [Table/Fig-8]. Mean serum osteoprotegerin levels were 10.85pmol/L and were the highest (11.45pmol/L) in 11 subjects (37%) who had normal BMD [Table/Fig-5]. BMD estimated 8(27%) patients to be osteoporotic, 11(37%) osteopenic and 11(37%) patients with normal BMD [Table/Fig-5].

Receiver-Operating Characteristic (ROC) Curve Analysis: Using BMD (range: 0 to -2.5) as the gold standard for

Parameter	Overweight	Obese	Morbidly Obese
Perimenopausal: N= 29 (72.5%)			
Number(Percentage) W: H RATIO >0.9 BMD: Normal Osteopenia Osteoporosis	N=24 15 (62.5%) 6 (25%) 8 (33.3%) 10 (41.7%)	N=4 2 (50%) 1 (25%) 1 (25%) 2 (50%)	N=1 1 (100%) 1 (100%) - -
POSTMENOPAUSAL: N=24 (60%)			
Number(Percentage) W: H RATIO >0.9 BMD: Normal Osteopenia Osteoporosis	22 15 (68%) 0 3 (14%) 19 (86%)	2 1 (50%) 0 1 (50%) 1 (50%)	(NIL) - - - -
NORMAL: N=21 (70%)			
Number(Percentage) W: H RATIO >0.9 BMD: Normal Osteopenia Osteoporosis	15 13 (87%) 6 (40%) 6(40%) 3 (20%)	6 5 (83%) 2 (33.3%) 2(33.3%) 2 (33.3%)	- -

[Table/Fig-7]: Distributions of various parameters with abnormal BMI in the three groups.

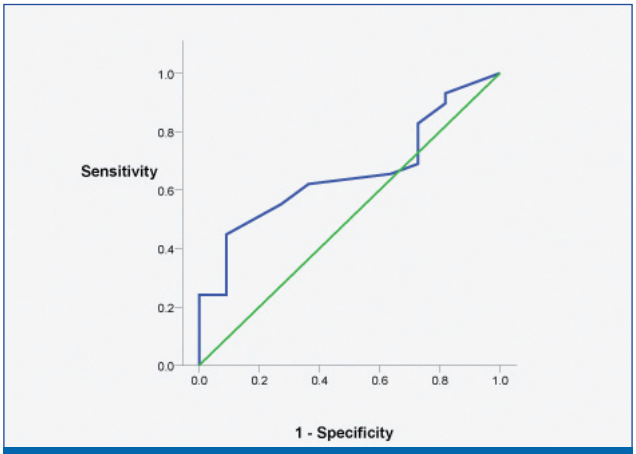
Parameters	Normal	Abnormal
Perimenopausal subjects with increased W:H ratio (n=21)		
Body Mass Index BMD	3 (14%) 4 (19%)	18 (86%) Overweight: 15 (83%) Obese 02 (11%) Morbidly obese 01 (6%) Osteopenia 05(24%) Osteoporosis 12(57%)
Postmenopausal subjects with abnormal Waist-Hip ratio (N=24)		
Body Mass Index BMD	8 (33%) 1 (4%)	16 (67%) Overweight 15 (94%) Obese 01 (6%) Morbidly obese 0 Osteopenia 07 (29%) Osteoporosis 16 (67%)
Normal subjects with abnormal Waist-Hip ratio: (N=19)		
BMI BMD	1 (5%) 6 (32%)	18 (95%) overweight 13 (72%) obese 05 (28%) morbidly obese 0 osteopenia 08 (42%) osteoporosis 05 (26%)

[Table/Fig-8]: Distribution of various parameters in subjects with increased W: H ratio.

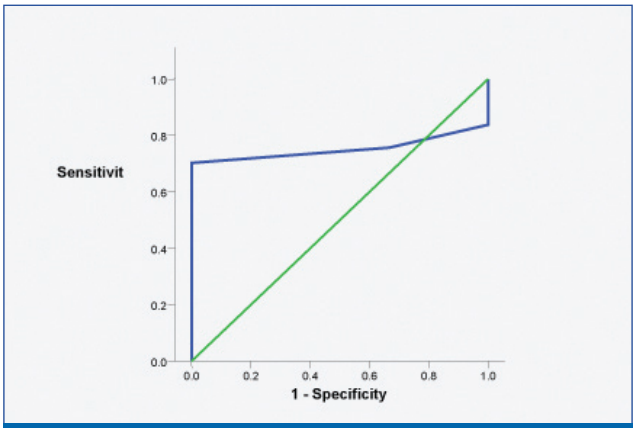
diagnosing osteoporosis, cut-off of OPG concentration was calculated which could differentiate between normal and osteoporotic women in the three groups. Serum OPG concentrations of 8.9pmol/L was able to predict osteoporosis in perimenopausal women. The predictive value of positive test was 69.57% and predictive value of negative test was 35.29% [Table/Fig-9,10]. The cut-off OPG concentration of 17.9pmol/l was able to predict osteoporosis in the postmenopausal

Parameter to be predicted	Cut-off value	Sensitivity	Specificity	Area under the curve
Osteoporosis				
Normal Group	10.32pmol/L	25%	43%	0.117
Group A	8.9 pmol/L	60%	46%	0.655 [Standard Error: 0.117 (p<0.134)]
Group B	17.9 pmol/L	54%	60%	0.150 [Standard Error: 0.071 (p<0.150)]

[Table/Fig-9]: Cut-off values of Serum Osteoprotegerin for predicting osteoporosis and complications



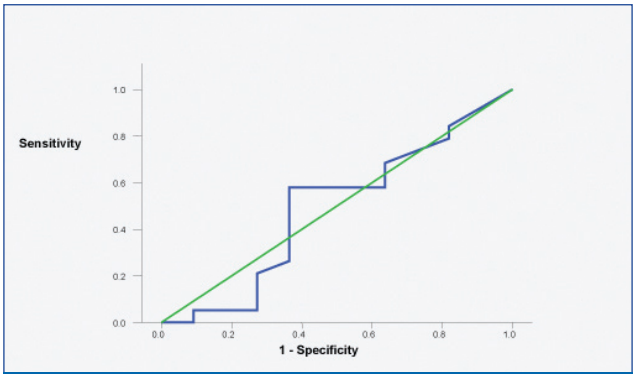
[Table/Fig-10]: ROC curve analysis for cut-off value of serum OPG for osteoporosis in perimenopausal women.



[Table/Fig-11]: ROC curve analysis for cut-off value of serum OPG for osteoporosis in postmenopausal women.

group with a sensitivity of 54% and specificity of 60%. The predictive value of positive test was found to be 90.48% and predictive value of negative test was 15.79% [Table/Fig-9,11]. In the normal group, a concentration of 10.32 pmol/l was able

to predict osteoporosis. The predictive value of the test was low (33%) as there were a high number of false positives and false negatives [Table/Fig-9,12].



[Table/Fig-12]: ROC curve analysis for cut-off value of serum OPG for osteoporosis in normal group.

DISCUSSION

The developmental, homeostatic and pathologic roles of Osteoprotegerin/RANK/RANKL pathway plays a pivotal role in controlling cell differentiation and function and are triggered by inflammatory cytokines or by disorders of calcium-phosphate homeostasis [1,5,11]. These interactions become evident in osteoporosis and vascular calcifications which are commonly associated with high morbidity and mortality in women [8,9].

To the best of our knowledge, this is the first case-control study conducted on the Indian female population. A normal group of women were included in the study as Indian standards are not available. In other studies, subjects were Chinese, Japanese, or Korean with most studies being conducted on Caucasian women [8-18]. Most researchers investigated namely two groups: premenopausal and the post-menopausal group [10,13,15,18-20]. In few other studies postmenopausal or the perimenopausal group were investigated along with men [11,12,16-18,21,22]. In comparison to the present exclusion criteria's, with that of other studies which only included male sex and black women because of their relatively low incidence of osteoporotic fractures [15].

There was a significant difference between the mean age ($p<0.001$) (range: 20-70 years) in the three groups which helped us to establish serum OPG levels in each group, without any significant overlap [Table/Fig-1].

This is the first case control study that has stratified body mass index into three categories i.e., overweight, obese and morbidly obese.Overall, the number of subjects found to be overweight (n=61) were more than the obese (n=12) or morbidly obese (n=1). The perimenopausal group had maximum number of patients with altered BMI (n=29), along with largest number of overweight (n=24) subjects. This finding can be attributed to the alteration in the circulating sex steroids during the perimenopausal transitional years which leads to altered BMI. The overweight subjects in all the three groups showed maximal derangement of parameters of CVD risk (BMI; W:H), with these subjects showing osteopenia and osteoporosis. Thus, due to altered BMI, the increased circulating lipoproteins available in the blood affect oestrogens levels and the state of bone health will be eventually decided by Osteoprotegerin levels. In the present study, no significant correlation of Body mass index was found with OPG in normal and perimenopausal women which shows that OPG levels are independent of body markers in these groups. In group B women, positive significant correlations ($p<0.005$) were found which may be because of the decrease in female sex hormones with age that leads to altered BMI and BMD but other investigators have reported no correlation [13,21,23-25].

The waist hip ratio had asignificant positive correlation with OPG only in postmenopausal women ($r=0.48$, $p<.002$). We could not compare the above finding with other researchers and only two investigators have reported significant association of W:H with OPG in women of all age groups [Table/Fig-1,6] [14,26].

The prevalence of osteoporosis is best measured by the frequency of reduced BMD. Standard X-rays do not provide an early assessment of fracture risk as 30-40% of bone must be lost before radiographic changes become apparent. Though the Dual Energy X-ray Absorptiometry (DEXA) of the hip and spine is the most widely used technique to measure bone mass accurately, QCT for bone density measurements can be performed [Table/Fig-2-4] [3]. In relation with BMD the correlation of age and BMI was significant ($p<0.001$) and maximum patients with osteoporosis were postmenopausal (24) [Table/Fig-5]. Patients with normal BMD and osteopenia were equally encountered in the normal and perimenopausal groups. This in keeping with the other studies shows that there is decrease in bone mineral density as age progresses. According to our present study 60% of post-menopausal and 40% of peri-menopausal patients had developed osteoporosis due to low oestrogen levels, in accordance with the menopausal status [Table/Fig-5]. Pammer A et al., reported negative correlation of BMD with OPG and postulated a higher risk of fractures in patients with osteoporosis. Dai YI et al., Browner WS et al., Holecki M et al., and Frost ML et al., reported no correlation between OPG and BMD at lumbar spine, which collaborated with our study [12,15,20,22,27].

Mean serum Osteoprotegerin levels increased with age and were highest in the post-menopausal group B (15.36 pmol/L). However, in total 110 patients, 48 women with osteoporosis showed elevated levels of OPG (mean=17.95pmol/L), while 37 osteopenic women had the lowest levels (8.90pmol/L) [Table/Fig-5]. As elevated serum OPG levels were found to increase with age and it may be hypothesised that this offers protection to the bone from further osteoclastic activity as OPG acts as

a decoy receptor to prevent interactions of the RANK-RANKL system thereby modulating osteoclastogenesis. Hence, rise in OPG levels is a compensatory protective mechanism in response to bone loss which occurs in the oestrogen deficient state with age, while osteopenia occurs because of decrease in levels of OPG below normal values [8,28]. Uemura H et al., in his cross-sectional study found serum OPG concentrations had significant positive correlations with age in 80 Japanese postmenopausal women (r=0.29, p=0.03) [10]. Similarly, Khosla S et al., found that serum OPG levels increased with age (r= 0.18, p<0.01) and were higher in the postmenopausal (1957pg/mL) [29]. The present findings i.e., increasing levels of serum OPG with age is in conformity with previous investigators [13-16,23,24,26,28]. Similarly, Rogers A et al., concluded that the variation in circulating estradiol levels is an important factor determining bone turnover and bone density at menopause and also suggested that circulating OPG levels may reflect its activity and are related to levels of estradiol [9].

Perimenopausal women showed a significant rise in OPG levels which may indicate the 'cardio-protective' nature of Osteoprotegerin. In the postmenopausal group, there was a significant rise in BMI and OPG levels with age (p<0.05) along with a significant correlation of OPG with age, BMI and waist-hip ratio(p<0.005). Serum OPG was found to be significantly raised in relation to the parameters of CVD risk [14]. Approximately the same serum levels (15.36±7.88 pmol/L) are also reported by Rogers A et al., Browner WS et al., and Kiechl S et al.. [9,15,16]. The rise of OPG levels during menopause can be attributed to up-regulation of OPG by oestrogen from osteoblasts, thus oestrogen deficiency during menopause results in increased bone remodelling. OPG levels rise in response to activation of several bone remodelling units and this is a compensatory mechanism to check osteoclastic activity.

In the normal group, mean OPG concentration was lower than that of the other groups, while BMI had a positive and very highly significant correlation with waist-hip ratio [Table/Fig-6]. This finding suggests that the correlation of BMI to W:H ratio is of less use in the transitional perimenopausal state.

Every test or assay done must fulfil the purpose of being utilized for the screening of the population or diagnosing a disease for therapeutic purposes [Table/Fig-13]. Thus, the present study attempts to establish a cut-off value of serum Osteoprotegerin in the three study groups which could be used in diagnosing osteoporosis and such a value would be age-specific [Table/Fig-9]. A serum Osteoprotegerin concentration of10.32 pmol/L was able to differentiate between women with normal bone density and women with osteoporosis in the normal group with low sensitivity and specificity which may be due to the fact that OPG is produced by a variety of tissues. In the perimenopausal group A, Serum OPG concentration less than 8.9 pmol/L were able to predict osteoporosis in perimenopausal women. In postmenopausal women serum concentration of 17.9 pmol/L was able to predict osteoporosis with a sensitivity of 54% and specificity of 60% and can be used as a marker for predicting osteoporosis in postmenopausal women. Few other studies showing an increase in serum OPG have attempted to associate the menopausal status with bone mineral density [9,12,15,19]. Liu JM et al., in their analysis found that there was no significant correlation between serum OPG and BMD, as was shown in our study [13]. Rogers A et al., and Browner WS et al., were also unable to find a significant correlation between OPG and BMD or overall fracture risk [9,15]. As per our knowledge only one other investigator has used the ROC curve analysis to show the prognostic value of serum osteoprotegerin levels [26].

LIMITATION

Several of the findings are in accordance with previous investigators, yet there are differences in our data from others. The stringent exclusion criteria along with removal of diseased subjects during the course of the present study, severely limited the number of subjects. Differences in the study design and population characteristics are likely to have contributed to the variability of results when compared with other studies. The final experimental results are related to validity of the products, operation skills of the end users and the experimental environments. Variability in the interactions

Peri/ Post-menopausal women											
Biophysical parameters (BMI;W:H)											
Normal						Altered					
Bone Mineral Density						Bone Mineral Density					
Normal		Decreased				Normal		Decreased			
Osteoprotegerin						Osteoprotegerin					
N	I	D	N	I	D	N	I	D	N	I	D
-	monitor	future risk	no active bone resorption	active disease	at present risk	-	presently protected	future risk	inactive disease	active disease	at risk

[Table/Fig-13]: Schematic representation of management of menopausal women in relation to Bone Mineral Density & Serum Osteoprotegerin levels.
N:Normal; I:Increased; D: Decreased

of OPG and its receptor RANKL may also lead to alterations in the circulating levels of OPG.

CONCLUSION

The levels of serum osteoprotegerin increases with age and it provides a compensatory protective mechanism to protect the skeletal system from osteoclastic damage. The mean serum OPG levels along with biophysical profile can be used as a biomarker for assessing osteoporosis in menopausal women. Though serum OPG bears no correlation with bone mineral density, it may be used as a screening test for osteoporosis to predict the burden of disease. This may aid the monitoring, treatment and prevention of osteoporosis.

REFERENCES

[1] Vogt MT, Valentin RS, Forrest KY, Nevitt MC, Cauley JA. Bone mineral density and aortic calcification: the Study of Osteoporotic Fractures. J Am Geriatr Soc. 1997;45(2):140-45.

[2] Whitehead MI. Menopause in. DK Edmond (6edn), Dewhursts textbook of obstetrics & gynaecology for postgraduates, New Delhi: Blackwell science Inc, 1999.

[3] Speroff L, Glass R, Kase N. Menopause and the perimenopausal transition. (7edn), Clinical gynaecologic endocrinology and infertility, New Delhi: Lippincott, Williams &Wilkins, 2004.

[4] Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, et al. Identity of Osteoclastogenesis Inhibitory Factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology. 1998;139(3):1329-37.

[5] Tsuda E, Goto M, Mochizuki SI, Yano K, Kobayashi F, Morinaga T, et al. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem Biophys Res Commun. 1997;234(1):137-42.

[6] Simonet WS, Lacey DL, Dunstan CR, Kelley MC, Chang MS, Luthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell. 1997;89(2):309-19.

[7] Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, et al. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. Biochem Biophys Res Commun. 1998;247(3):610-15.

[8] Kiechl S, Schett G, Schwaiger J, Seppi K, Eder P, Egger G, et al. Soluble receptor activator of nuclear factor-kappa B ligand and risk for cardiovascular disease. Circulation. 2007;116(4):385-91.

[9] Rogers A, Saleh G, Hannon RA, Greenfield D, Eastell R. Circulating estradiol and osteoprotegerin as determinants of bone turnover and bone density in postmenopausal women. J Clin Endocrinol Metab. 2002;87(10):4470-75.

[10] Uemura H, Yasui T, Kiyokawa M, Kuwahara A, Ikawa H, Matsuzaki T, et al. Serum osteoprotegerin/osteoclastogenesis-inhibitory factor during pregnancy and lactation and the relationship with calcium-regulating hormones and bone turnover markers. Journal of Endocrinology. 2002;174(2):353-59.

[11] Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, et al. Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. J Bone Miner Res. 1999;14(4):518-27.

[12] Dai Y, Shen L. Relationships between serum osteoprotegerin, matrix metalloproteinase-2 levels and bone metabolism in postmenopausal women. Chin Med J. 2007;120(22):2017-21.

[13] Liu JM, Zhao HY, Ning G, Zhao YJ, Chen Y, Zhang LZ, et al. The relationships between changes of serum osteoprotegerin, nuclear factor-kappa B ligand receptor activator, and age, menopause, bone biochemical markers and bone mineral densities in women aged 20-75. Zhonghuaneike Za Zhi. 2004;43(6):447-50.

[14] Oh ES, Rhee EJ, Oh KW, Lee WY, Baek KH, Yoon KH, et al. Circulating osteoprotegerin levels are associated with age, waist-to-hip ratio, serum total cholesterol, and low-density lipoprotein cholesterol levels in healthy Korean women. Metabolism. 2005;54(1):49-54.

[15] Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. J Clin Endocrinol Metab. 2001;86(2):631-37.

[16] Kiechl S, Schett G, Wenning G, Redlich K, Oberhollenzer M, Mayr A, et al. Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease. Circulation. 2004;109(18):2175-80.

[17] Naves M, Rodriguez-Garcia M, Diaz-Lopez JB, Gomez-Alonso C, Cannata-Andia JB. Progression of vascular calcifications is associated with greater bone loss and increased bone fractures. Osteoporos Int. 2008;19(8):1161-66.

[18] Naylor KE, Rogers A, Fraser RB, Hall V, Eastell R, Blumsohn A. Serum osteoprotegerin as a determinant of bone metabolism in a longitudinal study of human pregnancy and lactation. J Clin Endocrinol Metab. 2003;88(11):5361-65.

[19] Bezerra MC, Calomeni GD, Caparbo VF, Gebrim ES, Rocha MS, Pereira RM. Low bone density and low serum levels of soluble RANK ligand are associated with severe arterial calcification in patients with Takayasu arteritis. Rheumatology. 2005;44(12):1503-06.

[20] Fahrleitner-Pammer A, Dobnig H, Piswanger-Soelkner C, Bonelli C, Dimai HP, Leb G, et al. Osteoprotegerin serum levels in women: correlation with age, bone mass, bone turnover and fracture status. Wien Klin Wochenschr. 2003;115(9):291-97.

[21] Jono S, Ikari Y, Shioi A, Mori K, Miki T, Hara K, et al. Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease. Circulation. 2002;106(10):1192-94.

[22] Holecki M, Zahorska Markiewicz B, Janowska J, Nieszporek T, Wojaczynska Stanek K, Zak Golab A, et al. The influence of weight loss on serum osteoprotegerin concentration in obese perimenopausal women. Obesity. 2007;15(8):1925-29.

[23] Gannage-Yared MH, Yaghi C, Habre B, Khalife S, Noun R, Germanos-Haddad M, et al. Osteoprotegerin in relation to body weight, lipid parameters insulin sensitivity, adipocytokines, and C-reactive protein in obese and non-obese young individuals: results from both cross-sectional and interventional study. Eur J Endocrinol. 2008;158(3):353-59.

[24] Stern A, Laughlin GA, Bergstrom J, Barrett-Connor E. The sex-specific association of serum osteoprotegerin and receptor activator of nuclear factor kappaB legend with bone mineral density in older adults: The Rancho Bernardo study. Eur J Endocrinol. 2007;156(5):555-62.

[25] Gannage Yared MH, Fares F, Semaan M, Khalife S, Jambart S. Circulating osteoprotegerin is correlated with lipid profile, insulin sensitivity, adiponectin and sex steroids in an ageing male population. Clin Endocrinol. 2006;64(6):652-58.

[26] Anand DV, Lahiri A, Lim E, Hopkins D, Corder R. The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subjects. J Am Coll Cardiol. 2006;47(9):1850-57.
[27] Frost ML, Grella R, Millasseau SC, Jiang BY, Hampson G, Fogelman I, et al. Relationship of calcification of atherosclerotic plaque and arterial stiffness to bone mineral density and osteoprotegerin in postmenopausal women referred for osteoporosis screening. Calcified Tissue International. 2008;83(2):112-20.
[28] Grigorie D, Neacsu E, Marinescu M, Popa O. Circulating osteoprotegerin and leptin levels in postmenopausal women with and without osteoporosis. Rom J Intern Med. 2003;41(4):409-15.
[29] Khosla S, Arrighi HM, Melton LJ, Atkinson EJ, O'fallon WM, Dunstan C, et al. Correlates of osteoprotegerin levels in women and men. Osteoporos Int. 2002;13(5):394-99.

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