doi: http://dx.doi.org/10.5704/MOJ.1803.006

Role of Serum Osteoprotegerin as a Diagnostic Indicator of Primary Osteoporosis in Perimenopausal and Postmenopausal Women: An Indian Perspective

Pandey A, MS Ortho, **Khan YA,** MS Ortho, **Kushwaha SS,** MS Ortho, **Mohammed F,** MS Ortho, **Verma A,** MS Ortho

Department of Orthopaedics, ERA's Lucknow Medical College and Hospital, Lucknow, India

Date of submission: 4th April 2017
Date of acceptance: 9th February 2018

ABSTRACT

Introduction: Osteoporosis (OP) is a major health problem in the older population. The aim of the study was to assess the role of serum osteoprotegerin (OPG) as a diagnostic indicator of primary osteoporosis in peri- and post-menopausal women in an Indian population.

Materials and Methods: After ethical approval, 90 cases (45 cases and 45 controls) of peri- and post-menopausal females above 40 years of age presenting to our outpatient department were included in the study. It was a case controlled study conducted between July 2014 to July 2015. Based on the clinical features, t-score and radiological evidence from the DEXA scan, they were equally divided into two groups (cases and controls). Serum osteoprotegerin (OPG) levels were measured amongst cases and controls.

Results: The total calcium (mg/dl) level was lower among the cases and the difference was significant (p-Value= <0.001). Similarly, alkaline phosphatase (u/l), osteoprotegerin (u/ml) levels were higher in the cases as compared to controls and the difference was significant (p-Value= <0.001). The mean osteoprotegerin level showed a slight increase with increase in severity of the grading of BMD of spine. The results suggested a cut-off value of ≥10.5 u/ml (86.7% sensitive and 80% specific with accuracy of 84.5%) between normal and osteoporosis.

Conclusion: From the present study, we conclude that osteoprotegerin is a valid biomarker to diagnose postmenopausal women with low bone mineral density.

Key Words:
osteoporosis, osteoprotegerin, biomarkers

INTRODUCTION

Osteoporosis (OP) is a major health problem among the older adults, particularly in older women. It affects millions of people throughout the world and its frequency increases with age. Osteoporosis is characterized by an abnormally low bone mass and defect in bone architecture leading to increased bone fragility and chances of fracture[1]. At the cellular level, the bone tissues in the adults undergo a continuous process of remodelling in which the bone resorbing cells (osteoclasts) remove the old bone and bone forming cells (osteoblasts) replace the old bone with newly synthesized bone[2]. When the resorption is more than the formation, bone density is reduced and micro-architecture is disturbed leading to osteoporosis and increased bone fragility and chances of fracture[3]. The most common method employed to diagnose and categorize osteoporosis is bone mineral density (BMD) in different locations. World Health Organization (WHO) defines osteoporosis as bone mineral density (BMD) measured by dual energy radiograph absorptiometry (DEXA) scan less than -2.5 standard deviation below the mean value for young adults for same age and sex (T-score). Based on bone mineral density (BMD) osteoporosis is further classified as osteopenia, osteoporosis and severe osteoporosis[4]. Till now the bone strength prediction and fracture risk are mainly based on densitometric measurements. Recently various bone turns over markers have been identified to assess bone turnover rate which can also be used to monitor osteoporosis treatment.

In the year 1997, a few research groups identified a protein, named osteoprotegerin (OPG)[5-7]. Protein osteoprotegerin belongs to the tumour necrosis factor receptor (TNFR) family and is produced by osteoblasts. It is also produced by

Corresponding Author: Sudhir Shyam Kushwaha, Department of Orthopaedics, Department of Orthopaedics, ERA's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh, India
Email: sudhirshyamkushwaha@gmail.com

other cells like peripheral blood lymphocytes[8-10]. Osteoprotegerin acts as a soluble decoy for the receptor activator for nuclear factor K B Ligand (RANKL)[6]. It has already been proved that osteoprotegerin inhibits apoptosis by binding to the tumour necrosis factor (TNF) associated ligand (TRAIL, tumour necrosis factor related apoptosis inducing ligand)[11]. Many recent studies have proved the importance of the osteoprotegerin / RANK / RANKL system in the development of bone diseases[12]. The relationship between bone turnover markers (BTM) and the osteoprotegerin / RANK / RANKL system has not been fully established yet. Several studies demonstrated the direct and inverse correlation between the osteoprotegerin / RANKL and bone mineral density (BMD)[13,14].

We believe that there is no study done on the role of osteoprotegerin on primary osteoporosis in peri- and post-menopausal women in India. The aim of the study was to assess the role of serum osteoprotegerin (OPG) as a diagnostic indicator of primary osteoporosis in peri- and post-menopausal women in an Indian population in Lucknow, Uttar Pradesh, India.

MATERIALS AND METHODS

The study was conducted in the Department of Orthopaedics and Biochemistry in ERA's Lucknow Medical College and Hospital, Lucknow. It was a case controlled study conducted between July 2014 and July 2015. After ethical approval from the institutional ethics committee, 90 patients were recruited into the study. The study patients included 90 peri- and post-menopausal women. Based on the clinical features, t-score and radiological evidence from the DEXA scan, they were divided into two groups, Cases (n = 45) and Controls (n = 45). Females above 40 years of age with t-score below -1 SD were selected as cases and females with t-score above -1 SD were selected as controls. Females below 40 years of age, pregnant women, female with secondary osteoporosis, bone tuberculosis, liver disorders, alcoholism, thyroid and parathyroid disorders, women on medication with corticosteroids and heparin were excluded from the study.

Patients were recruited from the outpatient clinic of the Department of Orthopaedics. History taking and thorough physical examination and relevant investigations wherever required were done to exclude secondary osteoporosis. The recruited patients were informed of the purpose and relevance of the study. Those who agreed were included in the study after informed and written consent. All the patients were subjected to DEXA (three point) scan. Those whose t-score was more than -1 SD were taken into the control group and the others into cases. There were 45 cases and 45 controls. Observing aseptic precautions, 5ml whole venous blood sample of the recruited cases and controls was drawn and centrifuged. The serum was separated and stored in small capped vials for long term use at -20^{0}C until tested. Serum calcium, phosphorus and alkaline phosphatase levels were obtained. Serum osteoprotegerin level was measured by the ELISA kit, following instructions in the technical bulletin supplied along with the kit. After obtaining the result, serum osteoprotegerin levels were compared with the BMD and the relationship was assessed.

Proximal femur (neck, trochanter) and total hip regions and lumbar spine (L2–L4 region) BMD measurements (g/cm^2) were obtained by dual energy radiograph absorptiometry (DEXA) with the use of a lunar DPX (GE medical system). The interpretation of BMD was done as t-score according to WHO criteria: t-score of 1.0 as normal, t-score between -1.0 to -2.5 as osteopenia and t-score <-2.5 osteoporosis.

Serum osteoprotegerin was estimated by using the ELH-OPG-1 human-OPG-ELISA kit [RayBiotech Inc., Norcross, USA] according to the manufacturer's instructions. 100 µl standard or sample was added to each well and incubated for 2.5 hours at room temperature or overnight at 40ºC. After that 100 µl prepared biotin antibody was added to each well. After one hour incubation at room temperature, 100 µl streptavidin solution was added. After 45 minute incubation and the last washing step, the remaining conjugate was allowed to react with the substrate H_2O_2-tetramethylbenzidine (TMB). 100 µl TMB one-step substrate reagent was added to each well. The reaction was stopped by addition of acidic solution and absorbance of the resulting yellow product was measured at 450 nm.

Data was collected, revised, verified, edited and analysed statistically using SPSS statistical package (version 20.0). Descriptive statistics of all variables were presented as percentage; mean±SD. Statistical analysis was performed by describing the demographic characteristics of study participants. T-tests were used to test differences in the distribution of continuous variables, and the Chi-square test was used to test for differences in the distribution of categorical variables. Diagnostic validity of osteoprotegrin in osteoporosis was observed by receiver operating characteristic curve (ROC curve).

RESULTS

Comparative demographics as well as clinical parameters of the members of both cases as well as control groups are shown in Table I. On perusal of the demographic parameters (age, weight, height, BMI,) of the members of both the groups, we found no significant difference between the members of the two groups which made the two groups comparable. Mean age since last menstruation was higher among the cases and the difference was significant. As regards to the clinical parameters, the total calcium (mg/dl) level was lower among the cases and the difference was significant (p-Value=<0.001). Similarly, alkaline phosphatase (u/l), and osteoprotegrin (u/ml) levels were higher in the cases as compared to controls and the difference was significant (p-Value = <0.001) (Table I).

Table I: Shows distribution of different parameters in cases and control group

	Control (n=45) (p-Value)	Cases (n=45) Mean±SD	Significance Mean±SD
Age (year)*	57.40±9.57	57.58±9.35	0.930
Age since last menstruation (year)*	4.34±2.8	9.95±5.4	<0.001
Weight (kg)*	60.47±16.22	65.22±17.19	0.181
Height (cm)*	154.07±7.44	156.22±8.25	0.197
BMI*	25.04±4.53	26.31±4.59	0.192
Marital status			
Married#	35 (77.78%)	39 (86.67%)	
Unmarried#	4 (8.89%)	1 (2.22%)	0.35
Widow#	6 (13.33%)	5 (11.11%)	
Total calcium (mg/dl)*	9.64±0.49	8.3±1.91	<0.001
T-score			
Lumbar spine*	1.20±0.83	-3.20±1.29	<0.001
Femoral neck*	-0.56±0.50	-1.38±0.98	<0.001
Total hip*	1.56±1.09	-1.22±0.73	<0.001
Alkaline phosphatase (u/l)*	57.93±5.12	79.00±4.08	<0.001
Osteoprotegerin (u/ml)*	9.17±2.91	13.49±2.89	<0.001

*= value express in mean±standard deviation, # = value express in frequency (%)

Table II: Shows the distribution of osteoprotegerin (u/ml) level between BMD grading

	BMD grading of spine						
Osteo-protegerin (u/ml)	Normal* (n=45)	Low bone bone mass (osteopenia)* (n=15)	Mild osteoporosis* (n=8)	Moderate Osteoporosis* (n=17)	Severe osteoporosis (n=5)	F-value	p-Value
Mean (SD)	9.17(2.91)	11.20(2.09)	12.50(1.90)	13.80(2.11)	14.20(2.2)	13.67	<0.001

*= a normal value (the t score was more than -1), osteopenia (the t score was less than -1 and more than -2.5), mild (t score -2.5 through -3), moderate (t score -3.1 through -4), or severe (t score <or=-4.1)

Table III: Receiver Operator Characteristic (ROC) curve of osteoprotegerin (u/ml) of cases

Area under the curve Test result variable(s): osteoprotegerin (u/ml)				
Area	Std. Error[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
.845	.045	.000	.757	.933

The test result variable(s): osteoprotegerin (u/ml) has at least one tie between the positive actual state group and the negative actual state group.
A. Under the nonparametric assumption
B. Null hypothesis: true area = 0.5

The mean of osteoprotegerin level in the 45 controls was 9.17±2.91 u/ml. Among cases group, there were 15 (33.3%) patients with low bone mass, eight (17.8%) with mild, 17 (37.8%) with moderate and five (11.1%) with severe osteoporosis. The mean osteoprotegerin level showed a slight increase with increase in severity of grading of BMD of spine. [χ^2= 38.223; p<0.001] (Table II).

Receiver operator curve (ROC) analysis was done based on the direction of assessment. Osteoprotegerin level was evaluated for prediction of cut-off values between control group and cases group. The results suggested a cut-off value of ≥10.5 u/ml (86.7% sensitive and 80% specific with accuracy of 84.5%) between control group and cases group (Fig. 1).

The results of the Pearson's correlation test showed a significant inverse correlation between the serum osteoprotegerin (u/ml) concentration and BMD i.e. The total hip (r = -0.568, p<0.01), lumbar spine (r = -0.588, p<0.001), femoral neck (r = -0.347, p<0.05) and total calcium (r = - 0.331,p<0.01) (Table III).

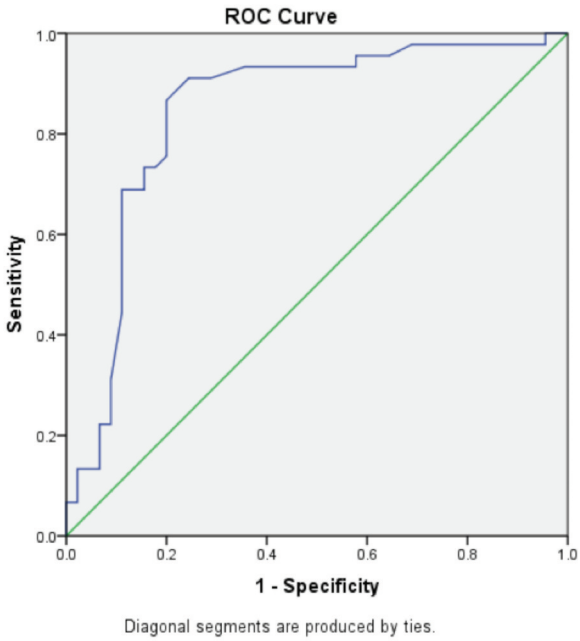

Fig. 1: Receiver Operator Characteristic (ROC) curve of osteoprotegerin of cases.

DISCUSSION

Osteoporosis is a major worldwide public health problem which causes significant morbidity, mortality, and socioeconomic burden[15]. It is defined as a disorder of skeletal system which leads to high risk of fragility fractures due to low bone strength. Maximum bony weakness occurs in peri- or post-menopausal women and is associated with insufficiency of oestrogen, a menopausal condition[16].

Higher circulating osteoprotegerin levels are often found in patients with osteoporosis and are usually regarded as a reflection of the increased bone turnover and a compensatory response to excessive osteoclast activity[17-19]. Hence in the present study, serum osteoprotegerin was significantly increased in cases (osteoporotic patients) than the control patients and showed a significant inverse correlation between serum osteoprotegerin concentration and bone mineral density of hip, lumbar spine and femoral neck.

Similarly, Youssef *et al* expressed results of osteoprotegerin as mean±SD using students t-test. Serum osteoprotegerin was significantly increased in reduced bone mineral density group ($P \leq 0.001$) when compared to the normal bone mineral group[20]. This was also supported by the work of Rogers *et al*, who stated that a significant negative correlation was observed between osteoprotegerin and BMD at total body, total hip, and femoral neck[21]. A study by Yano *et al* concluded that the osteoprotegerin serum levels were negatively correlated with bone mineral density at various sites (lumbar spine, femoral neck and total body) and positively correlated with biochemical markers of bone turnover[17].

Receiver operator curve analysis was done based on the direction of assessment. Osteoprotegerin (u/ml) was evaluated for prediction of cut-off values between control and cases. The results suggested a cut-off value of ≥10.5 u/ml (86.7% sensitive and 80% specific with accuracy of 84.5%) between normal and osteoporosis. Youssef *et al* found osteoprotegerin to be the most diagnostic bone marker to discriminate females with reduced bone mineral density from normal subjects. As regards high validity and overall accuracy, area under curve was 0.871, at cut off value ≤10.9 u/ml; serum osteoprotegerin showed 93.75% sensitivity and 91.7% specificity. The main limitations of the current study were small size of the studied sample, single centric study, and short period of the study and lacunae of literatures on osteoporosis in Indian patients using serum osteoprotegerin as a diagnostic indicator.

CONCLUSION

Osteoprotegerin is a valid biomarker to diagnose postmenopausal women with low bone mineral density. The results suggested a cut-off value of ≥10.5 u/ml (86.7% sensitive and 80% specific with accuracy of 84.5%) between normal and osteoporosis. This may suggest a new promising measure to early diagnose patients at high risk of low bone mineral density and subsequently giving early appropriate treatment.

ACKNOWLEDGEMENT

The authors acknowledge the support of the Department of Biochemistry, Era's Lucknow Medical College in conducting the study.

CONFLICT OF INTEREST

There was no conflict of interest in this study and no external funding was received.

REFERENCES

1. Armas LA, Recker RR. Pathophysiology of osteoporosis: new mechanistic insights. *Endocrinol Metab Clin North Am.* 2012; 41(3): 475-86.
2. Khosla S. Update in male osteoporosis. *J Clin Endocrinol Metab.* 2010; 95(1): 3-10.
3. Watts NB, Bilezikian JP, Camacho PM, Greenspan SL, Harris ST, Hodgson SF, *et al.* American Association of Clinical Endocrinologists Medical Guidelines for Clinical Practice for the diagnosis and treatment of postmenopausal osteoporosis. *Endocr Pract.* 2010; Suppl 3:1-37.
4. Kanis JA, Burlet N, Cooper C, Delmas PD, Reginster JY, Borgstorm F, *et al.* European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int.* 2008;19(4):399-428.
5. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, *et al.* Osteoprotegerin: a novel secreted involved in the regulation of bone density. *Cell.* 1997; 89(2): 309-19.
6. Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F, Morinaga T, *et al.* Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. *Biochem Biophys Res Commun. 1*997; 234(1): 137-42.
7. Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, *et al.* Identity of osteoclastogesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. *Endocrinology.* 1998; 139(3): 1329-37.
8. Chakravarti A, Marceau AA, Flamand L, Poubelle PE. Normal human primary CD4+ T lymphocytes synthesis and release functional osteoprotegerin in vitro. *Lab Invest.* 2008; 88(2): 171-84.
9. Ziolkowska M, Kurowska M, Radzikowska A, Luszcykiewicz G, Wiland P, Dziewczopolski W, *et al.* High levels of osteoprotegerin and soluble receptor activator of nuclear factor kappa b ligand in serum of rheumatoid arthritis patients and their normalization after anti-tumor necrosis factor alpha treatment. *Arthritis Rheum.* 2002; 46(7): 1744-53.
10. Vanderborght A, Linsen L, Thewissen M, Geusens P, Raus J, Stinissen P. Osteoprotegerin and receptor activator of nuclear factor-kappaB ligand mRNA expression in patients with rheumatoid arthritis and healthy controls. *J Rheumatol.* 2004; 31(8): 1483-90.
11. Anderson DM, Maraskovsky E, Bilingsley WL, Dougall WC, Tometsko ME, Roux ER, *et al.* A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature.* 1997; 390(6656): 175-9.
12. Theoleyre S, Wittrant Y, Tat SK, Fortun Y, Redini F, Heymann D. The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiological bone remodeling. *Cytokine Growth Factor Rev.* 2004; 15(6): 457-75.
13. Nabipour I, Larijani B, Vahdat K, Assadi M, Jafari SM, Ahmadi E, *et al.* Relationships among serum receptor of nuclear factor-kappaB ligand, osteoprotegerin, high-sensitivity C-reactive protein, and bone mineral density in postmenopausal women: osteoimmunity versus osteoinflammatory. *Menopause.* 2009; 16(5): 950-5.
14. Uemura H, Yasui T, Miyatani Y, Yamada M, Hiyoshi M, Arisawa K, *et al.* Circulating profiles of osteoprotegerin and soluble receptor activator of nuclear factor kappaB ligand in post-menopausal women. *J Endocrinol Invest.* 2008; 31(2): 163-8.
15. Delmas PD, Fraser M. Strong bones in later life: luxury or necessity? *Bull World Health Organ.* 1999; 77(5): 416-22.
16. Aggarwal N, Raveendran A, Khandelwal N, Sen RK, Thakur JS, Dhaliwal LK, *et al.* Prevalence and related risk factors of osteoporosis in peri- and postmenopausal Indian women. *J Midlife Health.* 2011; 2(2): 81-5.
17. 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.
18. 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): 631-7.
19. Jabbar S, Drury J, Fordham JN, Datta HK, Francis RM, Tuck SP. Osteoprotegerin, RANKL and bone turnover in postmenopausal osteoporosis. *J Clin Pathol.* 2011; 64(4): 354-7.
20. Youssef EMI, Ewieda GH, Ali HAA, Tawfik AM, Ezzat AA, El-Khouly N, *et al.* Osteoprotegerin as a bone marker of osteoporosis and their relation with obesity and leptin. *Basic Sciences of Medicine.* 2012; 1(5): 46-53.
21. 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): 4470-5.