

# Bone Turnover Markers: An Emerging Tool to Detect Primary Osteoporosis

MAYUR RAI<sup>1</sup>, TIRTHAL RAI<sup>2</sup>, JANICE D'SA<sup>3</sup>, SRINIDHI RAI<sup>4</sup>

## ABSTRACT

**Introduction:** Osteoporosis is a global health problem, asymptomatic and noticed only after a fracture. Ten years after the cessation of ovarian function due to hormonal imbalance, women show an accelerated phase of reduction in bone mass known as primary osteoporosis. Determination of Bone Mineral Density (BMD) and Dual Energy X-Ray Absorptiometry (DEXA) are the gold standard diagnostic approaches for osteoporosis. However, they are costly; hence Bone Turnover Markers (BTMs) have been suggested to detect the risk of osteoporotic fractures.

**Aim:** To assess the potential of BTMs as a diagnostic tool for primary osteoporosis in order to reduce the associated morbidity and mortality.

**Materials and Methods:** This study was conducted on 105 women who were divided into Group I with 35 pre-menopausal women, Group II with 35 post-menopausal women without fractures and Group III with 35 post-menopausal women with wrist, spine or hip fractures. Serum osteocalcin (OC), calcium, phosphate, alkaline phosphatase, total protein, albumin and 24 hour urinary hydroxyproline were estimated. Statistical analysis

was done using Statistical Package for the Social Sciences (SPSS) Version 16.0. ANOVA test was done to compare variables among the groups. Tukey's test was applied for intergroup comparison. Student's t-test was used to compare two groups and correlation studies were done using Pearson's correlation. Statistical significance was considered as  $p < 0.05$ .

**Results:** The mean serum OC level in post-menopausal women with fractures was statistically low when compared to the pre-menopausal and post-menopausal women. The mean urinary hydroxyproline level was statistically higher in post-menopausal women with fractures and a positive correlation of urinary hydroxyproline was seen with age and BMI. The mean OC level was lowest in the spine fractures, when compared to the wrist and hip. Urinary hydroxyproline level had no significant difference among the fracture groups.

**Conclusion:** BTMs can be an inexpensive diagnostic and screening tool for primary osteoporosis. Low serum OC levels and high urinary hydroxyproline levels in post menopausal women could thus predict osteoporosis and its timely intervention could prevent the risk of osteoporotic fractures thereby decreasing the mortality rate.

**Keywords:** Alkaline phosphatase, Hydroxyproline, Osteocalcin, Pre-menopausal women, Post-menopausal women

## INTRODUCTION

Bone is a dynamic tissue that grows in size during childhood and accelerates its growth during adolescence. The peak bone mass is attained at the age of 35 years, after which it starts declining with increase in age. The normal bone density is defined as: 1 Standard Deviation (SD) or greater than the mean value in young adults of the same age and sex (t-score of  $-2.5$ ). Bone density  $< 2.5$  SD of peak bone mass and impaired bone quality is called osteoporosis [1].

Osteoporosis is a metabolic bone disease which involves reduction in bone mass and deterioration of the bone tissue micro architecture leading to enhanced bone fragility [2]. Initially, osteoporosis is asymptomatic and noticed only after a fracture. It is globally considered as a silent killer due to its propensity to cause fracture in the elderly age group. Primary osteoporosis is commonly seen in women after about 10 years of the cessation of ovarian function. The general prevalence of primary osteoporosis in India has been reported to be 42.5% among women aged 50 years and above [3]. It is also estimated that every 1 out of 5 males and 1 out of 3 females suffer from osteoporosis, making India one of the largest affected countries in the world [4].

The gold standard for diagnosing future osteoporotic hip and spine fractures are BMD and DEXA, which is unaffordable, and compression fractures of the vertebrae are difficult to detect as these are asymptomatic. As a result, the BTMs have recently come to trend in assessing the fracture risks. BTMs are protein derivatives released during bone remodelling [5]. The bone formation marker OC is synthesised predominantly by the osteoblasts and

incorporated into the extracellular matrix of bone. Osteocalcin is dependent on post translational carboxylation for its hydroxyapatite affinity. Bone remodelling is a continuous process, involving both bone formation and bone resorption, which in turn relies on the activities of osteoclasts (resorption), osteoblasts (formation) and osteocytes (maintenance).

In healthy adults, bone formation is coupled with bone resorption, wherein the rate of resorption is equal to the rate of formation which is regulated by various hormones and local mediators. An imbalance in bone turnover is seen especially in metabolic bone diseases such as osteoporosis, somatic growth, ageing and other conditions affecting bone turnover. This imbalance is primarily due to uncoupling of bone resorption and formation, where both processes may not occur efficiently at the same time. Therefore, osteocalcin serves as a valid marker to detect dynamic changes in bone turnover, both when formation and resorption are coupled as well as uncoupled [6,7].

The traditional resorption marker measures the collagen degradation products from osteoclasts. Hydroxyproline is derived from proline that undergoes post-translational hydroxylation to form collagen, a major component of the bone. The hydroxylysine glycosides are integral parts of bone collagen and occur in two forms: glycosyl-galactosyl hydroxylysine (Glc-Gal-Hyl) and galactosyl-hydroxylysine (Gal-Hyl). Both components are released into the circulation during collagen degradation and can be measured in urine [8].

The aim of the present study was to assess the difference in the levels of BTMs in various menstrual stages of women and its applicability

as a diagnostic tool for primary osteoporosis in order to substantially reduce the morbidity and mortality in the study population.

## MATERIALS AND METHODS

The cross-sectional study was conducted on 105 women visiting the Department of Orthopaedics and Gynaecology at AJ Institute of Medical Science & Research Centre, Mangalore from July 2011–December 2012, after approval from the Institution Ethical Committee (AJEC/2009/34). The current mean and SD obtained for serum osteocalcin among three groups (35 subjects in each group) was applied in G\*power (statistical software), which yielded a minimum sample size of 15 (5 in each group). Alpha error being 5%, Power being 95% and effect size 1.25. The subjects were divided into three groups which included:

- Group I: consisting of 35 pre-menopausal women.
- Group II: consisting of 35 post-menopausal women without fractures.
- Group III: consisting of 35 post-menopausal women with fractures (sample was collected on the day of the fracture) Fractures were sustained as a result of trivial trauma involving the wrist, spine and hip.

Exclusion criteria involved subjects with chronic illness, kidney and liver disorder, diabetes, surgically induced menopause, patients on hormonal therapy, steroidal therapy, subjects having fractures as a result of road traffic accident and pregnant women.

An informed consent was obtained from all the study participants. 5 mL of venous blood sample was collected in a plain vacutainer and centrifuged at 4000 rpm. Serum was then analysed for OC, alkaline phosphatase (ALP), calcium, phosphorous, total protein and albumin. A 24-hour urine sample was also collected and taken after adding a preservative (HCl: 5 mL/L of urine) to analyse urinary hydroxyproline.

### Methods of Estimation

Estimation of serum calcium was done by Arsenazo method [9], inorganic phosphorous by phosphomolybdate method [10], ALP by International Federation of Clinical Chemistry (IFCC)/kinetic method [11], total protein by biuret method [12] and albumin by bromocresol green method [13] using Agappe reagent kits on LabLife RoboChem autoanalyser. Serum OC was estimated by chemiluminescent method [14] in autoanalyser Siemens' Immulite 1000 and urinary hydroxyproline by modified Neuman and Logan method [15] in a spectrophotometer using commercially available kits.

## STATISTICAL ANALYSIS

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) version 16.0. Categorical variables were represented as frequency and percentage. Continuous variables were represented as mean±SD. ANOVA test was done to compare variables among three groups. Intergroup comparison was done using Tukey's test. Comparison study between the variables of two groups was done using student's t-test. Karl Pearson's test correlation was used to correlate the data. Statistical significance was considered as  $p < 0.05$ .

## RESULTS

The study group consisted of 35 pre-menopausal, 35 post-menopausal and 35 osteoporotic post-menopausal women. The age group of the study participants ranged from 20 to 80 years

with the mean age (in years) being 28.37±5.28, 57.09±7.16 and 68.34±8.13 in group I, II and III respectively.

The mean BMI was 18.68±2.40 kg/m<sup>2</sup> in osteoporotic post-menopausal women as compared to the post-menopausal women (22.32±2.28 kg/m<sup>2</sup>) and pre-menopausal women (18.68±2.40 kg/m<sup>2</sup>). This difference was statistically very highly significant ( $p < 0.001$ ). A significant decrease was observed in group III to group I and group III to group II ( $p < 0.05$ ).

The mean serum OC levels in group III, II and I were 2.79±2.33, 12.61±4.34 and 5.68±2.83 ng/mL respectively, which was statistically significant ( $p$ -value  $< 0.001$ ). OC levels had significantly decreased from group II to group I, group II to group III and group I to group III ( $p < 0.05$ ).

Mean urinary hydroxyproline was statistically higher in group III (27.88±6.67 mg/dL) when compared to the other two groups ( $p < 0.001$ ). It was interesting to note a statistically higher mean serum ALP in post-menopausal women with fractures (105±44.36 U/L) when compared to the other two groups. The mean serum calcium level was lowest in group III (8.69±0.64 mg/dL) ( $p < 0.001$ ) [Table/Fig-1].

Parameter	Group I (n=35)	Group II (n=35)	Group III (n=35)
Age (years)	28.37±5.28	57.09±7.16 a*	68.34±8.13 b*c*
BMI (kg/m <sup>2</sup> )	21.50±2.03	22.32±2.28	18.68±2.40 b*c*
Age of attaining menarche (years)	12.71±0.57	12.40±1.04	12.57±0.98
Age of attaining menopause (years)	-	48.74±2.72	48.26±2.85
Time since menopause (years)†	-	8.34±6.27	20.09±8.38
S. Calcium (mg/dL)	9.32±0.64	9.17±0.65	8.69±0.64 b*c*
S. Phosphate (mg/dL)	2.79±0.57	2.78±0.55	2.95±0.86
S. ALP (U/L)	75.00±10.06	95.75±20.63 a*	105.26±44.36 b*
S. Total Protein (g/dL)	6.53±0.41	6.51±0.32	6.31±0.56
S. Albumin (g/dL)	3.99±0.23	4.03±0.18	3.65±0.38 b*c*
S. Osteocalcin (ng/mL)	5.68±2.83	12.61±4.34 a*	2.79±2.33 b*c*
Urinary Hydroxyproline (mg/dL)	5.73±2.53	16.66±3.32 a*	27.88±6.67 b*c*

**[Table/Fig-1]:** Comparison of clinical and biochemical parameters between the study groups.  
 BMI: Body Mass Index; ALP: Alkaline Phosphatase; a: comparison between Group I and Group II; b: comparison between Group I and Group III; c: comparison between Group II and Group III; \*:  $p < 0.05$ ; level of significance obtained from Tukey's test (post-hoc analysis/intergroup comparison); †: Age minus Menopause Age

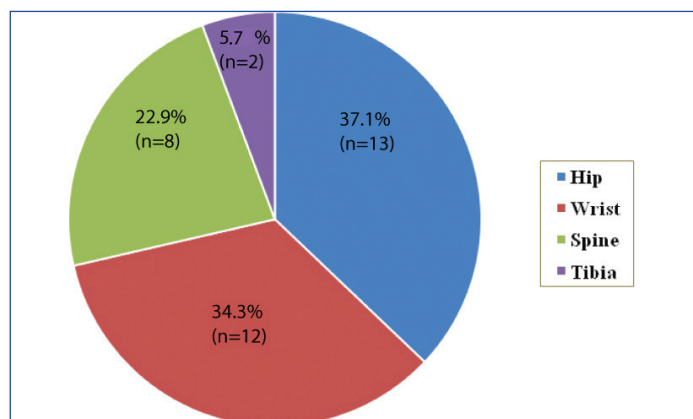
Correlation studies considering all three groups revealed a highly significant ( $p < 0.001$ ) positive correlation of serum OC with BMI ( $r = 0.484$ ) and albumin ( $r = 0.379$ ). Urinary hydroxyproline exhibited a statistically significant strong positive correlation with age ( $r = 0.806$ ,  $p < 0.001$ ) and an inverse correlation with BMI ( $r = -0.441$ ) and calcium ( $r = -0.341$ ) ( $p < 0.001$ ) [Table/Fig-2].

Group III was further divided according to the fracture types in which 37.1% ( $n = 13$ ) had hip fractures, 34.3% ( $n = 12$ ) wrist fractures, 22.9% ( $n = 8$ ) spine fracture and 5.7% ( $n = 2$ ) tibia fracture [Table/Fig-3]. The significance of BTMs were analysed on the different fracture types except tibia due to a very small number. Overall, serum OC was higher among subjects with wrist fracture (2.44±0.34 ng/mL). A statistically high significant difference was present in the mean

Variables	Pearson's correlation (r)								
	Age	BMI	Serum Calcium	Serum Phosphorus	Serum ALP	Serum Total Protein	Serum Albumin	Serum Osteocalcin	Urinary Hydroxyproline
Serum Osteocalcin	-0.080	0.484**	0.184	-0.106	-0.031	0.089	0.379**	1.000	-0.139
Urinary Hydroxyproline	0.806**	-0.441**	-0.341**	0.182	0.479**	-0.194*	-0.392**	-0.139	1.000

**[Table/Fig-2]:** Correlation of bone markers with various parameters in all study subjects ( $n = 105$ ).  
 BMI: Body Mass Index; ALP: Alkaline Phosphatase; \*  $p < 0.05$ , level of significance obtained from Pearson's correlation test; \*\*  $p < 0.001$ , level of significance obtained from Pearson's correlation test

serum osteocalcin values between the wrist fractures and spine fractures ( $p < 0.05$ ), although the urinary hydroxyproline level was not significantly different in the various fracture groups [Table/Fig-4].



**[Table/Fig-3]:** Distribution of fracture types among post-menopausal women with fracture (Group III).

Parameter	Hip Fracture (n=13)	Wrist Fracture (n=12)	Spine Fracture (n=8)
S. Calcium (mg/dL)	8.48±0.65	8.63±0.49	9.13±0.78
S. Phosphorus (mg/dL)	2.78±0.94	2.96±0.87	3.25±0.84
S. ALP (U/L)	119.46±61.23	87.17±20.36	117.13±33.23
S. Total Protein (g/dL)	6.09±0.62	6.50±0.33	6.55±0.57
S. Albumin (g/dL)	3.43±0.42	3.77±0.23	3.84±0.42a*
S. Osteocalcin (ng/mL)	2.19±0.31	2.44±0.34	2.01±0.02b*
Urinary hydroxyproline (mg/dL)	27.29±6.89	26.67±5.54	32.16±7.23

**[Table/Fig-4]:** Biochemical parameters based on various fracture types in post-menopausal women with fracture.

ALP: Alkaline Phosphatase; a: comparison between spine fracture and hip fracture; b: comparison between spine fracture and wrist fracture; \*:  $p < 0.05$ , level of significance obtained from Tukey's test (post-hoc analysis/ intergroup comparison)

## DISCUSSION

Detection of osteoporosis in post-menopausal women at an early stage, before development of bone fractures is essential. Hence, the efficacy of BTMs on the entire bone and its effect on various factors causing osteoporosis needs to be extensively studied. In this study, we observed very low levels of serum OC and high levels of urinary hydroxyproline excretion in osteoporotic post-menopausal women as compared to the women in other two groups. A study conducted in Sweden, also shows 20% lower OC levels among the post-menopausal women with fractures than healthy post-menopausal women. Similar findings were also seen in a study by Pietschmann P et al., [16]. However, the present study findings were not in accordance with the study carried out by Kalaiselvi VS et al., which reported a high level of OC in osteoporotic post-menopausal women compared to non osteoporotic women [17].

Present study showed increased urinary hydroxyproline excretion in osteoporotic women in comparison to post-menopausal women without fractures, which was similar to the study findings of Jagtap VR et al., [18] and a pilot study done by Rai T et al., [19].

Pearson correlation coefficient showed a positive correlation of urinary hydroxyproline and negative correlation of OC to age in this study which is in parallel to a study done by Das M et al., [20]. In late menopause, bone formation is reduced and resorption activity is increased which is reflected by decrease in OC levels and increase in urinary excretion of hydroxyproline, which indicates the degradation of type I collagen of bone matrix thus explaining the propensity to fractures in these women even with a trivial fall. At the molecular level, it can be explained on the basis of deficiency of oestrogen, which accelerates the receptor activator of nuclear factor kappa B ligand (RANKL) that decreases osteoporosis [20]. However, hydroxyproline is found in other tissues and can be liberated in

their abnormalities. In early menopause, there is an accelerated osteoclastic activity due to the sudden depletion of oestrogen. This increases the bone resorption and as bone re-modelling is coupled, serum OC and urinary hydroxyproline increases.

Decreased BMI is directly proportional to low BMD causing osteoporotic fractures in older women as shown in this study where osteoporotic post-menopausal women had significantly low mean BMI as compared to the women in the other two groups and had a positive significant correlation with serum OC. This is supported by a study done by Morin S et al., which concluded that low bone mass, body weight or visceral fat and low OC can predict osteoporosis, making serum OC a highly sensitive bone formation marker [21]. Sri Rekha P et al., similarly showed a significant negative correlation of urinary hydroxyproline and BMI [22] and inverse results was seen in a study by Paneri S et al., [23].

Exhaustion of oestrogen in menopause has deleterious effects on the health of menopausal women and one of these is due to increased excretion of calcium and decreased absorption in the intestine. In the present study, we observed low mean serum calcium level in osteoporotic women as compared to the other groups and serum calcium showed a positive correlation with serum OC. A significant negative correlation was seen with urinary hydroxyproline which was in parallel to the results obtained by Prabha YS et al., [24].

Serum ALP is a bone formation marker and is present in various other tissues. In our study, it showed a negative correlation with serum OC which has the same action and a significant positive correlation with urinary hydroxyproline. Increase in serum ALP may also occur in osteoporotic women due to intake of certain drugs affecting liver enzymes or systemic response of the body to injury following a fracture. Therefore, serum ALP is a non-specific bone formation marker. These findings were consistent with a study by Bowles SA et al., [25].

Albumin is a major component of protein and a sensitive marker of poor nutritional status. This study showed statistically lower serum albumin levels in osteoporotic post-menopausal group and showed a positive correlation with OC. Similarly a longer duration of lower serum albumin levels was observed in a few studies, which made the patient susceptible to fractures [26]. The mechanism of hypo-albuminemia in osteoporosis remains controversial. It could be either due to albumin being present in the osteoid or it may directly cause changes in bone remodelling with release of NF- $\kappa$ B which activates osteoclasts and decreases osteoblasts. Hence, explaining its positive significant correlation with serum OC and negative significant correlation with urinary hydroxyproline.

Serum OC was statistically lower in vertebral fractures as compared to wrist fractures and no statistical difference was seen among the spine and hip fractures which is in accordance with a study done by Soroush M et al., who showed that the mean urinary hydroxyproline levels had no statistical difference in the three types of fracture [27].

## LIMITATION

A small sample size restrained authors from finding the exact cut-off value of the BTMs in each fracture type and the effect of BTMs on different osteoporotic treatment modalities could have been evaluated.

## CONCLUSION

Bone turnover markers can be an inexpensive diagnostic and screening tool for primary osteoporosis. Low serum OC levels and high urinary hydroxyproline levels in post-menopausal women could thus predict osteoporosis and its timely medical intervention could prevent the risk of osteoporotic fractures thereby decreasing the morbidity and mortality.

## REFERENCES

- [1] Singh S, Kumar D, Lal AK. Serum osteocalcin as a diagnostic biomarker for primary osteoporosis in women. *J Clin Diagn Res.* 2015;9(8):RC04-07.
- [2] Consensus A. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. *Am J Med.* 1993;94(6):646-50.
- [3] Thulkar J, Singh S. Overview of research studies on osteoporosis in menopausal women since the last decade. *Journal of mid-life health.* 2015;6(3):104-07.
- [4] Jagtap VR, Ganu JV, Nagane NS. BMD and serum intact osteocalcin in post-menopausal osteoporosis women. *Indian Journal of Clinical Biochemistry.* 2011;26(1):70-73.
- [5] Kling JM, Clarke BL, Sandhu NP. Osteoporosis prevention, screening and treatment: a review. *Journal of Women's Health.* 2014;23(7):563-72.
- [6] Seibel MJ. Biochemical markers of bone turnover part I: biochemistry and variability. *The Clinical biochemist. Reviews/ Australian Association of Clinical Biochemists.* 2005;26(4):97-122.
- [7] Atalay S, Elci A, Kayadibi H, Onder CB, Aka N. Diagnostic utility of osteocalcin, under carboxylated osteocalcin, and alkaline phosphatase for osteoporosis in premenopausal and post-menopausal women. *Annals of Laboratory Medicine.* 2012;32(1):23-30.
- [8] Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomarker Research.* 2017;5(1):18.
- [9] Bauer PJ. Affinity and stoichiometry of calcium binding by arsenazo III. *Analytical Biochemistry.* 1981;110(1):61-72.
- [10] Teitz NW, ed. Estimation of serum inorganic phosphorous. *Clinical Guide to Laboratory Tests, 3rd Ed.* Philadelphia; WB Saunders, 1995.
- [11] Schumann G, Klauke R, Canalias F, Bossert-Reuther S, Franck PF, Gella FJ, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37°C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline phosphatase. *International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division, Committee on Reference Systems of Enzymes (C-RSE) (1).* *Clin Chem Lab Med.* 2011;49(9):1439-46.
- [12] Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry.* 1949;177(2):751-66.
- [13] Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta.* 1971;31(1):87-96.
- [14] Merle B, Delmas PD. Normal carboxylation of circulating osteocalcin (bone Glaprotein) in Paget's disease of bone. *Bone Miner.* 1990;11(2):237-45.
- [15] Leach AA. Notes on a modification of the Neuman & Logan method for the determination of the hydroxyproline. *Biochemical Journal.* 1960;74(1):70.
- [16] Pietschmann P, Resch H, Krexner E, Woloszczuk W, Willvonseder R. Decreased serum osteocalcin levels in patients with post-menopausal osteoporosis. *Acta medica Austriaca.* 1991;18(5):114-16.
- [17] Kalaiselvi VS, Prabhu K, Ramesh M, Venkatesan V. The association of serum osteocalcin with the bone mineral density in post-menopausal women. *J Clin Diagn Res.* 2013;7(5):814-16.
- [18] Jagtap VR, Ganu JV. Effect of antiresorptive therapy on urinary hydroxyproline in post menopausal osteoporosis. *Indian J Clin Biochem.* 2012;27(1):90-93.
- [19] Rai T, Rai M, Kumar A, Shetty M, Rai S, Prajna. Effect of menopause on bone turnover. *World J Pharm Sci.* 2014;2(8):775-79.
- [20] Das M, Biswas UK, Kumar A. 24 hours urinary hydroxyproline - a non-invasive, cost effective and early biochemical marker which may be used to screen the osteoporotic lesion in post-menopausal women. *Asian J Med Sci.* 2015;6(4):21-24.
- [21] Morin S, Tsang JF L, Leslie WD. Weight and body mass index predict bone mineral density and fractures in women aged 40 to 59 years. *Osteoporosis Int.* 2009;20(3):363-70.
- [22] Sri Rekha P, Venkateswarlu U, Sarada U. Comparative study of biochemical bone turnover markers in pre and post-menopausal women. *Int J Appl Res.* 2015;1(5):185-87.
- [23] Paneri S, Panchonia A, Varma M, Dey Sarkar P, Yadav S. Impact of body mass index and low estrogen level on urinary hydroxyl proline and other bone related parameters in post-menopausal women. *Int J Reprod Contracept Obstet Gynecol.* 2014;3(2):335-37.
- [24] Prabha YS, Ashalata K, Babu PV, Kumari PK, Nagamani M. A Study of bone markers (serum calcium, serum phosphorus and serum alkaline phosphatase) in post menopausal women in East Godavari District, Andhra Pradesh, India. *IOSR Journal of Dental and Medical Sciences.* 2015;14(6):01-03.
- [25] Bowles SA, Kurdy N, Davis AM, France MW, Marsh DR. Serum osteocalcin, total and bone-specific alkaline phosphatase following isolated tibial shaft fracture. *Annals of Clinical Biochemistry.* 1996;33(3):196-200.
- [26] Afshinnia F, Wong KK, Sundaram B, Ackermann RJ, Pennathur S. Hypoalbuminemia and osteoporosis: reappraisal of a controversy. *The Journal of Clinical Endocrinology.* 2016;101(1):167-75.
- [27] Soroush M, Khabbazi A, Malek Mahdavi A. Serum osteocalcin levels in post-menopausal osteoporotic women receiving alendronate. *Rheumatology Research.* 2018;3(2):83-89.

### PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Orthopaedics, A.J Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India.
2. Associate Professor, Department of Biochemistry, K.S Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India.
3. Assistant Professor, Department of Biochemistry, A.J Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India.
4. Assistant Professor, Department of Biochemistry, K.S Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India.

### NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Tirthal Rai,  
Associate Professor, Department of Biochemistry, K.S Hegde Medical Academy, Nitte University,  
Deralakatte-575018, Mangalore, Karnataka, India.  
E-mail: tirthalrai@nitte.edu.in

Date of Submission: **Jul 18, 2018**  
Date of Peer Review: **Aug 28, 2018**  
Date of Acceptance: **Oct 06, 2018**  
Date of Publishing: **Dec 01, 2018**

FINANCIAL OR OTHER COMPETING INTERESTS: None.