Original Article-Orthopaedics

Comparison of Bone Turnover Markers on Osteoporosis in Pre and Postmenopausal Women

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Abstract

Osteoporosis, a major public health problem, is becoming increasingly prevalent with the aging of the world population. Rapid bone loss occurs in postmenopausal women due to hormonal factors which lead to increased risk of fractures. Biochemical markers of bone metabolism are used to assess skeletal turnover. Bone formation markers (Total Calcium, Ionised calcium,Alkaline phosphatase), and bone resorption markers (Urinary Hydroxyproline) were analysed in pre and post menopausal women. A cross-sectional study of 200pre- and post menopausal women was carried out at Sree Balaji Medical College and Hospital,chennai. Bone formation markers (Total Calcium, Ionised calcium, Alkaline phosphatase), and bone resorption markers (Urinary Hydroxyproline) were analysed in pre and post menopausal women. Bone formation markers, Total and Ionised calcium were significantly decreased ($p<0.001$) and Alkaline phosphatase was significantly increased ($p<0.001$) in postmenopausal women compared to premenopausal women. Bone resorption markers, Urinary hydroxyproline excretion was significantly increased ($p<0.001$) in postmenopausal women. The results from this study suggests that simple, easy, common biochemical markers such as age, years after menopause,urinary hydroxyproline, total serum ALP, total serum calcium and ionized calcium could be used as indicators of increased bone turnover, to enable early intervention so as to minimize fracture due to osteoporotic changes.

Key Words: *Osteoporosis,Bone turnover, Alkaline phosphatase, Hydroxyproline, Osteopenia*

Introduction

The word 'menopause' is derived from two Greek words, 'meno' (month) and 'paus' (to stop). Clinically, menopause is said to have occurred when menstruation has ceased for twelve months [(1)]. Physiologically, menopause is defined as the permanent cessation of menses resulting from reduced ovarian hormone secretion that occurs naturally or is induced by surgery, chemotherapy, or radiation [(2)]. The post-menopausal stage in women is essentially an oestrogen-deficient state [(1)]. Both menopause and aging are associated with an accelerated loss of bone mass. Menopause occurs when the balance between bone formation and resorption is upset and resorption is excessive, resulting in a negative remodelling balance [(3).]

Biochemical markers of bone turnover have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone disease [(4)]. They reflect the whole body rates of bone resorption (Resorption markers) and bone formation (Formation markers) .Therefore they may provide a more representative index of the overall skeletal bone loss than would be obtained by measuring the rates of change in Bone Mineral Density (B.M.D) at specific skeletal sites [(5).] Osteoporosis is

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more common in post-menopausal women and not only gives rise to morbidity but also markedly diminishes the quality of life in this population. There is lack of information regarding the risk factors of osteoporosis in developing countries [6]. The occurrence of Osteoporosis in postmenopausal women is very common problem especially in India who are exposed to many of the risk factors like Family h/o osteoporosis, history of anorexia or bulimia, prolonged amenorrhea, low calcium diet, lack of exercise, Vitamin D deficiency. But there are very few Indian studies regarding the prevalence of osteoporosis in postmenopausal women and also regarding the biochemical markers which indicate bone turnover in our setup.

Osteoporosis is an important public health problem in middle-aged and older women. Until recently, the diagnosis and monitoring of treatment for osteoporosis has been confined to clinical assessment, radiography and bone densitometry. However, in recent years biochemical markers of bone formation and resorption have been developed to quantify bone turnover and remodelling, with possible applications in clinical practice. Osteoporosis is a major health and economic problem. An International consensus development conference has stated that osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitect deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. This silently progressing metabolic bone disease is widely prevalent in India, and osteoporotic fractures are a common cause of morbidity and mortality in adult Indian men and women [7]. Osteoporosis has been linked to an increased fracture risk and subsequent mortality in the later life. Previous prediction models have focused on osteoporosis in postmenopausal women; however, a prediction tool for osteopenia is needed. Osteoporosis is a serious global health issue due to the rapid increase in the size of the aging population [5-6] Osteoporosisrelated fractures have been found to be associated with significant costs, increased morbidity and mortality,reduced quality of life, and loss of independence [5-6].The prevalence of osteoporosis increases with age for all sites, and by WHO definition up to It is important to think clearly about the 2 principle determinants in adult bone health (a) Maximum attainment of Peak bone mass (PBM) in young adulthood, and (b) the rate of bone loss with advancing age. With the onset of menopause, rapid bone loss occurs which is believed to average approximately 2% to 3% over the following 5 to 10 yrs, being greatest in the early postmenopausal years [7]. Life time losses may reach 30% to 40% of the peak bone mass in women and 20% to 30% in men. The pathogenesis of postmenopausal osteoporosis involves the interplay of many factors- Nutritional,Environmental, Genetic factors [8].

Total alkaline phosphatase activity in serum has been used commonly as a biochemical marker of osteoblast function, but lacks specificity because of the contribution of activity derived from the liver, in particular. Human alkaline phosphatases (ALP) are a group of enzymes of similar specificity coded for by at least four different gene loci that catalyse the hydrolysis of phosphate esters at an alkaline pH [9-11].The gene for tissue non-specific ALP encodes the isoenzyrnes expressed in liver, bone and kidney. In healthy individuals about half the activity of alkaline phosphatase in serum is derived from bone and the remainder from liver. The isoforms differ only in the degree of sialylation and glycosylation, reflected in differences in electrophoretic mobility, heat stability and precipitation by lectin. Methods to separate and quantify bone ALP in the presence of liver ALP, based on these properties, have not had sufficient specificity or sensitivity to be useful clinically. In the USA, the prevalence of osteoporosis in postmenopausal women aged 50 years has been found to be 15.8% in non-Hispanic whites, 7.7% in non Hispanic blacks, and 20.4% in Mexican Americans [12]. Among postmenopausal women, the average bone mineral density (BMD) was found to be highest among African Americans,followed by among Hispanics, native Americans, and Asians [13]. In Taiwan, the prevalence of osteoporosis among women aged 40 years has been estimated at 10.1% and 7.5% based on the BMD of the spine and femoral neck,respectively [14]. Despite the availability of diagnostic tools and treatment protocols, osteoporosis remains underdiagnosed and undertreated [15]. Women reach peak bone mass between the ages of 20 years and 30 years. Then, BMD decreases gradually and continues to decline rapidly after menopause [16]. Therefore, predicting the risk of osteoporosis at an earlier age (e.g., premenopausal) or a preclinical phase (i.e., osteopenia) is crucial for early prevention of osteoporosis.

Osteoporosis is more common in post-menopausal women and not only gives rise to morbidity but also markedly diminishes the quality of life in this population. There is lack of information regarding the risk factors of osteoporosis in developing countries [17-18]. Serum alkaline phosphatase (ALP) is the most commonly used biomarker of bone formation. ALP is a ubiquitous enzyme that plays an important role in osteoid formation and bone mineralisation. The serum ALP pool consists of several dimeric isoforms that originate from various tissues, such as the liver, bone, intestine, spleen, kidney, and placenta [19-21]. Thus, the aim of the present study is to evaluate the risk of accelerated bone mass loss by assessing bone markers, such as alkaline phosphatase (ALP) and serum calcium, in post-menopausal women.

Therefore,a tool that includes easily accessible factors may be a plausible approach to predict the risk of osteopenia. Some biomarkers (e.g., blood or urine markers) can easily be obtained during routine health checkups and improve the sensitivity in predicting osteoporosis at the preclinical phase (i.e., osteopenia). However, biomarkers are rarely included in the currently available prediction tools. Therefore, this study aimed to develop a simple and accurate prescreening tool for identifying premenopausal and early postmenopausal women (aged 40-55 years) with a high risk of osteopenia via the incorporation of biomarkers.

Thus, the aim of the present study is carried out to assess the clinical utility of biochemical markers of bone turnover, such as Total Calcium, Ionised calcium, Alkaline phosphatase in pre- and post menopausal women and to evaluate the risk of accelerated bone mass loss by assessing bone markers, such as alkaline phosphatase (ALP) and serum calcium, in post-menopausal women

Material and Methods

We performed a cross-sectional study of 200 pre- and post menopausal women, at Department of Orthopaedics, *Sree Balaji Medical College and Hospital,chennai.* The Institutional Ethical Committee approved the study and Informed consent was obtained from each participant in the study. The study group consisted of 100Postmenopausal women in the age group of 46-65 years and 100 Premenopausal women in the age group of 25-45 years. All the participants were non smokers, non alcoholic and ambulatory. The women were neither pregnant nor on oral contraceptive pills. None of the postmenopausal women had suffered any fracture in the previous 1 year nor were they on Hormone replacement therapy or any other medication that might affect bone turnover. Based on time since menopause, 100 postmenopausal women were categorized into 2 groups. 30 women were in their early postmenopausal period (<5years) and remaining 70 women were in their late postmenopausal period (>5years). Height and Weight of all the participants were noted and Body mass index (BMI) was calculated using the formula = Weight (Kg) / Height2 (m).

5 ml of random blood sample was collected in a plain bulb from each participant. Serum was separated immediately by centrifuging at 2000rpm for 15min and analysed for Total calcium , Ionised Calcium and Alkaline phoshatase. Random sample of urine was collected at the same time in a clean plastic bulb and analysed for Hydroxyproline and Creatinine immediately.

Stastistical Analysis

The data were presented as Mean ± SD. Statistical analysis were done by using Microsoft Excel and SPSS for windows version 11.5 (SPSS, Inc., Chicago). P value <0.05 was considered statistically significant.

Results

Table. I**.** shows the comparison of all the biochemical parameters estimated as their mean values.

Table. I : Comparison of markers of bone turnover in pre- and post Menopausal women (values expressed as mean ± SD)

Parameters	Pre menopausal women(n=100)	Post menopausal women (n=100)
Age	33.32±6.16	57.80±7.93**
Years after Menopause		11.72±7.84
BMI	23.42±4.57	25.68±5.76*
Alkaline phosphatase	2.42±0.56	3.49±1.09**
T.Serum Calcium (m.mol/lit)	2.241±0.25	2.099±0.18**
I. Serum Calcium (m.mol/lit)	1.39±0.24	1.38±0.26**
Urinory Hydroxy Proline (mg /g crt)	10.48±3.28	22.82±10.96**

*p<0.01 (significant), **p<0.001 (highly significant), **(**mg /g crt=milly grams per grams creatinine**)**

Table. II : Comparison of markers of bone turnover in Early (<5yrs) and Late (>5 yrs) post menopausal period (values expressed as mean ± SD)

Parameters	Early menopausal Period (n=30)	Post menopausal Period (n=70)
Age	49.9±4.47	59.9±12.4**
Years after Menopause	3.18±1.53	14.93±4.36***
BMI	25.8±4.38	25.73±5.79*
Alkaline phosphatase	3.83±1.16	3.45±1.37*
T.Serum Calcium (m.mol/lit)	2.091±0.17	2.36±0.48**
I. Serum Calcium (m.mol/lit)	1.17±0.14	1.27±0.24*
Urinory Hydroxy Proline (mg /g crt)	25.27±13.78	21.33±14.36*

*(not significant), **p<0.05 , ***p<0.001 (highly significant)

Table. 2. shows the comparison of all the biochemical markers of bone turnover on early and late postmenopausal periods.

Discussion

Current guidelines from the US Preventive Services Task Force recommend that women younger than 65 years be screened for osteoporosis using dual-energy X-ray absorptiometry (DXA) if their 10-year risk of a major osteoporotic fracture is greater than or equal to that of a 65-year-old white woman without additional risk factors [(22)]. Biochemical parameters can give an idea as to the rates of bone formation and resorption. High rate of bone turnover correlates with a low bone mass. Calcium salts in bone are embedded in collagen fibrils, 13% of which is mainly hydroxyproline. During bone loss, collagen fibrils are broken down and hydroxyproline is thus excreted in the urine. Urinary hydroxyproline(OHPr) is thus considered as an index of bone resorption and a major determinant of bone status [(23)]. In this study urinary hydroxyproline is expressed as mg of hydroxyproline per gram of creatinine, because creatinine is excreted in the urine in relatively constant amounts proportional to an individuals muscle mass, thus serving as a reference standard [(23-24)]. In the present study there was a significantly increased urinary excretion of hydroxyproline (mg/g Cr) in postmenopausal women when compared to premenopausal women. Other potential uses of turnover markers include the ability to monitor drug efficacy, to predict increases in bone mass, and to assist in the selection of patients for treatment. Bone-turnover markers have little or no use in the diagnosis of osteoporosis, in the prediction of bone mass, and in the ability to monitor compliance.

Menopause is known to be associated with numerous physiological and biochemical changes affecting bone mineral metabolism. The results of various case control studies in pre- and post-menopausal women have shown that changes in the serum calcium levels in post-menopausal women are not statistically significant [(25-27)] ; however, in the present study, we found that the serum calcium levels were significantly reduced in the post-menopausal group ($p<0.001$) when compared to the pre-menopausal group ($p<0.0001$) Ashuma et al. reported that aging and menopause lead to a decline in oestrogen and progesterone production, which has been implicated in the increased calcium levels of post-menopausal women [(25)]. The response of markers of bone turnover may inform the physician about patient compliance,absorption of the bisphosphonate and the effect of treatment. It has been suggested that markers of bone turnover could be used to predict the response of an individual (in terms of BMD) to bisphosphonate therapy. In general, women lose about 1% of their bone density per year during and after menopause. However, nearly 35% of women lose bone at a faster rate during the late perimenopausal period. Biochemical markers can detect women who are considered “rapid losers” that is, those who lose 3% to 5% of bone per year [(28)].

The most common sources of elevated serum ALP levels are liver and bone. In bone, ALP is present on the cell surface of the osteoblasts and probably cleaved off from the membrane and released into circulation. In healthy individuals, about half of the serum ALP is derived from bone. Therefore, measurement of S-ALP, can be used as a bone turnover marker, but it lacks sensitivity and specificity, especially in conditions where there is only a small increase in bone turnover. Measurement of the bone-specific isoform, S-Bone ALP, has better sensitivity for detecting changes in bone turnover. However, currently available S-Bone ALP assays still have a crossreactivity of 15–20% with the liver isoenzyme. Serum alkaline phosphatase is the most commonly used marker of bone formation. ALP is a ubiquitous enzyme that plays an important role in osteoid formation and mineralization.The total ALP serum pool consists of several dimeric isoforms which originate from various tissues such as liver, bone,intestine, spleen, kidney and placenta. In adults with normal liver function, approximately 50% of the total ALP activity in serum is derived from the liver, whereas 50% arises from bone [(10)] . In our study the total ALP levels were significantly high in postmenopausal women in comparison to premenopausal women. Bonespecific ALP level is a bone turnover marker that is associated with low BMD and fracture risk [(29)]. A previous Taiwanese study showed that serum total and bone-specific ALP levels slowly increased in women after the age of 40 years [(30)].These two types of ALP have been found to have a linear association and a high level of correlation among people without bone diseases.[(30)] Therefore, total ALP serves as a surrogate for bone-specific ALP level. Finally, the number of years since menopause was an important predictor of osteopenia. ALP level reflects the physiological status in the body, which is more sensitive than using weight or age for predicting subtle changes in BMD during the preclinical phase of osteoporosis. a

serum marker, total ALP level, which can be obtained during routine health checkups, significantly increased the sensitivity of the model for osteopenia prediction compared with the existing tools. Early prediction of osteoporosis has important public health and clinical implications, as osteoporosis usually has no symptoms until the occurrence of fractures. Conversely, the serum alkaline phosphatase (ALP) levels were significantly increased in the post-menopausal group compared to the pre-menopausal group ($p<0.001$). It has also been shown that oestrogen deficiency, as occurs during menopause, induces the synthesis of cytokines by osteoblasts, monocytes, and T cells and thereby stimulates bone resorption by increasing osteoclastic activity. This action results in modification of the reabsorption, excretion, and resorption of calcium, which leads to increased circulating levels of this ion [31-34]. Thus, we have reported a negative correlation between serum calcium and ALP levels in post-menopausal women. Several studies have reported no significant correlation between serum calcium levels and ALP when assessing various years since menopause [35-40]. However, contrary to these findings, higher levels of calcium and ALP have been demonstrated in early post-menopausal women compared with late menopausal women ($p<0.001$) [34].

The ALP levels were high in women during their early postmenopausal period when compared to late postmenopausal period (Table II). Ionised calcium levels were found to be significantly decreased in Early postmenopausal women compared to late postmenopausal women.(Table II).This shows that the bone mass continues to decline with age but at a slower rate than during the early menopausal time. Annual change in ALP seems to indicate that bone resorption prevails on bone formation in early postmenopausal period. Total calcium and ionized calcium also the markers of bone formation were significantly decreased in postmenopausal women, when compared to premenopausal women.

Preanalytical variability is one of the limitations affecting the clinical interpretation of bone marker measurement. Level of markers are affected by diurnal, menstrual and seasonal variations, food intake, and the level of physical activity. Uncontrollable factors include for example age, gender, menopausal status, recent fracture, bed rest, metabolic bone disease, and renal function. Bone turnover markers are highest in early morning and lowest in late afternoon and evening. The largest diurnal variation has been reported for urinary collagen crosslinks and crosslinked telopeptides . Serum levels of bone turnover markers are less affected [40].

The results from this study suggests that simple, easy, common biochemical markers such as age, years after menopause, urinary hydroxyproline, total serum ALP, total serum calcium and ionized calcium could be used as indicators of increased bone turnover, to enable early intervention so as to minimize fracture due to osteoporotic changes. These markers may help identify women at greatest risk for bone loss who would benefit most from therapeutic interventions. To the best of our knowledge, the OPAT is the first tool developed for predicting osteopenia in women aged 40 to55 years. Integration of a serum marker, total ALP level,Calcium and urinary hydroxyproline which can be obtained during routine health checkups, significantly increased the sensitivity of the model for osteopenia prediction compared with the existing tools. More studies are warranted to validate this new tool for predicting osteopenia risk in women aged 40 to 55 years.

Although bone turnover markers have shown clinically interesting associations, currently available bone turnover markers also have some limitations. They reflect quantitative changes but do not provide information on structural abnormalities of bone or remodeling rate of different bone compartments. Some analytes have high variability or are not bone-specific. Recent progress in the identification of important pathways in bone physiology has led to the development of potential new biochemical markers. These include osteoclastic enzymes, regulators of bone cell activity, non collagenous matrix proteins, or their fragments, and markers of bone matrix properties.

Conclusion

Bone turnover markers, in particular those associated with bone resorption rate, have potential for clinical use in many applications related to skeletal disorders. Guidelines for their use are gradually becoming available. Novel, more specific markers as well as improvement and standardization of measurement techniques will enhance reliability and facilitate the use of bone turnover markers in practice. Therefore, an increase in

bone turnover accelerates bone mass reduction in post-menopausal women, whereas a decrease in bone turnover is associated with the preservation of bone mass.

In normal post-menopausal women, an increase in bone turnover accelerates bone mass reduction. The present study reveals that serum calcium levels are significantly reduced in post-menopausal women, whereas serum ALP levels are significantly increased. In addition, a significant negative correlation was observed between serum calcium and ALP levels in the experimental group. Measurement of bone turnover through urinary hydroxyproline,Alkaline phosphatase and Calcium could form a tool available to assist health care professionals to predict fracture risk. Early prediction of osteoporosis has important public health and clinical implications, as osteoporosis usually has no symptoms until the occurrence of fractures. *The results from this study suggest that simple, easy, common biochemical markers can still be used to assess the bone turnover in postmenopausal women and hence their risk of developing osteoporosis and fractures.*conclude that bone ALP is useful in monitoring alendronate treatment of patients with osteoporosis.It has better clinical utility for following both treatment groups and monitoring individual patients. The biochemical markers of bone turnover provide dynamic measures of bone remodeling and thus potentially useful in predicting the course of changes in bone mass. The inclusion of serum total alkaline phosphatase level in the model, which is easy to obtain from routine health checkups, significantly enhanced the sensitivity for detecting osteopenia in women aged 40 to55 years.

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References

1. Price CP, Thompson PW. The role of biochemical tests in the screening and monitoring of osteoporosis. *Ann Clin Biochem* 1995; 32: 244-60
2 Demers LM, Kleerekoper M. Recent advances in biochemical markers of bone turnover. *Clin Chern* 1994; 40: 1994-5
3 Blumsohn A, Eastell R. The performance and utility of biochemical markers of bone turnover: do we know enough to usc them in clinical practice'!*Ann Clin Biochem* 1997; 34: 449-59
4. Gupta A. Osteoporosis in India—the nutritional hypothesis.Natl Med J Ind 1996:9(6):268-74
5. Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. Lancet 2011;377:1276e87.
6. U.S. Department of Health and Human Services. Bone health and osteoporosis: a report of the surgeon general. Rockville,MD: U.S. Department of Health and Human Services; 2004.
7. Susan A. Calcium Supplementation in Postmenopausal Women. From Medscape Ob/Gyn & Women's health,2003:8(2) http://www.medscape.com/viewarticle/460438
8. Delmas PD. Biochemical markers of bone turnover for the clinical investigation of osteoporosis. Osteoporos Int 1993:3(1):81-6
9. Woitge HW, Nave CS, Kissing C, Bruckner GL, Meyer K,Grauer A, et al. Seasonal Variation of Biochemical Indexes of Bone Turnover; Results of a population-based Study. J Clin Endocrinol Metab 1998: 92(1): 68-75
10. Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J . The Use of Biochemical Markers of Bone Turnover in Osteoporosis. Osteoporos Int 2000: Suppl 6:2-17
11. Moss OW. Perspectives in alkaline phosphatase research. *Clin Chern* 1992; 38: 2486 92
12. Wright NC, Looker AC, Saag KG, Curtis JR, Delzell ES, Randall S,et al. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. J Bone Miner Res 2014;29:2520e6.
13. j G, Mendel A, Lam MA, Akhavan PS, Cancino-Romero J,Waugh E, et al. A clinical decision rule to enhance targeted bone mineral density testing in healthy mid-life women. Osteoporos Int 2012;23:1931e8.
14. Gitelman HJ. An Improved automatic procedure for the determination of Calcium in biologic specimens, Anal Biochem 1967:18: 521-31
15. Isselbacher KJ, Braunwad E, Wilson JD, Martin JB, Fauci AS, Kasper DL , editors. Harrison's Principles of Internal Medicine,13th Ed. Vol 2,Mc

Graw-Hill publication, 1994, 2139 pp

16. Daly JA, Ertingshausen G. Direct Method for Determining Inorganic Phosphate in Serum with the "CentrifiChem", Clin Chem 1972: 18(3): 263-5
17. Kanis JA, Johnell O, Oden A, Jonsson B, De Laet C, Dawson A.Risk of hip fracture according to the World Health Organization criteria for osteopenia and osteoporosis. Bone 2000;27:585e90.
18. Waugh EJ, Lam MA, Hawker GA, McGowan J, Papaioannou A,Cheung AM, et al. Risk factors for low bone mass in healthy 40e60 year old women: a systematic review of the literature. Osteoporos Int 2009;20:1e21.
19. Demir B, Haberal A, Geyik P, Baskan B, Ozturkoglu E,Karacay O, et al. Identification of the risk factors for osteoporosis among postmenopausal women. Maturitas 2008;60:253e6.
20. Jeon YK, Lee JG, Kim SS, Kim BH, Kim SJ, Kim YK, et al. Association between bone mineral density and metabolic syndrome in pre- and postmenopausal women. Endocr J 2011;58:87e93.
21. Sheng YH, Chen JH, Chiou JM, Tsai KS, Lee YY, Tsao CK, et al.Association of renal function and menopausal status with bone mineral density in middle-aged women. Sci Rep 2015;5:14956.
22. Civitelli R, Armamento-Villareal R, Napoli N. Bone turnover markers: understanding their value in clinical trials and clinical practice. Osteoporos Int 2009;20:843e51.
23. George BO. Urinary and Anthropometrical Indices of Bone Density in healthy Nigerian Adults. J Appl Sci Environ Mgt 2003:7(1):19-23
24. Justesen TI, Petersen JLA, Ekbom P, Damm P, Matheisen ER. Albumin-to-Creatinine ratio in random urine samples might replace 24-h urine collections in screening for Micro and Macroalbuminuria in pregnant women with Type-1 Diabetes. Diabetes Care 2006: 29(4):924-5.
25. Ashuma S, Shashi S, Sachdeva S. Biochemical Markers of bone turnover: diagnostic and therapeutic principles. *Osteoporosis.* 2005;**3**:305–311.
26. Suresh M. Naidu DM. Influence of years since menopause on bone mineral metabolism in south Indian women. *Indian J Med Sci.* 2006;60(5):190–198.
27. Massé PG, Dosy J, Jougleux JL, Caissie M, Howell DS. Bone mineral density and metabolism at an early stage of menopause when oestrogen and calcium supplement are not in used and without interference of major confounding variables. *J Am College Nutr.* 2005;**24**:354–360.
28. Rosen C J, Tenenhouse A. Biochemical markers of bone turnover. Postgraduate Medicine 1998:104(4):101-18.
29. Hsu SH, Tsai KS. Different age-related trends of bone and nonbone forms of alkaline phosphatase in Chinese men and women. Clin Chem 1997;43:186e8.
30. Jarava C, Armas JR, Salgueira M, Palma A. Bone alkaline phosphatase isoenzyme in renal osteodystrophy. Nephrol Dial Transplant 1996;3:43e6.
31. Esbrit P. Hyper calcemia of malignancy: New insights into an old syndrome. *Clin Lab.* 2001;47(1–2):67–71.
32. Riggs BL, Khosla S, Melton LJ. A unitary model of involutional osteoporosis: Oestrogen deficiency causes both type 1 and type 2 osteoporosis in postmenopausal women and contributes to bone loss in ageing men. *J Bone Miner Res*. 1998;13(5):763–773.
33. Kurland ES, Cosman F, McMahon DJ. Parathyroid hormone as a therapy for idiopathic osteoporosis in men. Effect on bone mineral density and bone markers. *J Clin Endocinol Meta.* 2000;85(9):3069–3076.
34. Sengupta P. The Laboratory Rat: relating its age with humans. *Int J Prev Med.* 2013;4(6):624–630.
35. Dutta S, Joshi KR, Sengupta P, Bhattacharya K. Unilateral and bilateral cryptorchidism and its effect on the testicular morphology, histology, accessory sex organs and sperm count in Laboratory Mice. *J Hum Repro Sci.* 2013;6(2):106–110.
36. Sengupta P. Potential Health Impacts of Hard Water. *Int J Prev Med*. 2013;4(8):866–875.
37. Sengupta P, Sahoo S. A Cross Sectional Study to Evaluate the Fitness Pattern among the Young Fishermen of Coastal Orissa. *Indian J Pub Health Res Dev.* 2013;4(1):171–175.
38. Sengupta P, Chaudhuri P, Bhattacharya K. Male Reproductive Health and Yoga. *Int J Yoga.* 2013;6 (2):87–95.
39. Sengupta P, Banerjee R. Environmental toxins:

Alarming impacts of pesticides on male fertility. *Hum Exp Toxicol*. 2013. Forthcoming 2014 Feb. doi: 10. 1177/0960327113515504.

40. Sengupta P. Health Impacts of Yoga and Pranayama: An Art-of-the-state Review. *Int J Prev Med*. 2012; 3(7):444–458.