Original Article

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Orthopaedics Section

The Role of Biochemical Markers in the Early Detection of Osteoporosis in Women: A Comparative Study from the Western Region of Nepal

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ABSTRACT

Introduction: Osteoporosis is defined as the reduced bone mass per unit volume of normal mineralized bone that leads to fractures, even with minor trauma. Osteoporotic fractures are a common cause of morbidity and mortality in adult men and women. This silently increasing metabolic bone disease is extensively prevalent in developing countries like Nepal. The objective of our study was to achieve an easy and early detection of osteoporosis in postmenopausal women, detecting the more vulnerable premenopausal women also.

Materials and Methods: This was a hospital based, comparative study which was carried out in the Department of Orthopedics of Manipal Teaching Hospital, Pokhara, Nepal, between 31st December 2009 and 31st July 2011. The variables which were collected were age (years), years after menopause (years), BMI (kg/m^2), total serum calcium (mmol/L), ionized calcium (mmol/L), phosphorus (mmol/l), total protein (g/dl), albumin (g/dl) and ALP (units/L). The approval for the study was obtained from the institutional research ethical committee.

Results: Of the 612 subjects, 306 were pre menopausal and the other 306 were postmenopausal women. The post menopausal women were further categorized into early (132) and late (174) post menopausal women. For all the subjects, the mean values and the p value was calculated with all variables which were taken into our study. There was no significant difference in the mean values of the BMI of the pre-menopausal (24.77± SD2.19) women and those of the post-menopausal women [(24.77± SD1.76) p value (0.99)]. The mean values of serum calcium were moderately reduced in post-menopausal women (2.05± SD0.11) as compared to those in the pre-menopausal women (2.22 ± SD0.20). These were found to be statistically significant (p value 0.001).

Conclusion: The bone turnover markers are a better way of the early detection of the high risk women and those in the early phases of osteoporosis when the X-ray and DEXA scan changes are not prominent.

Key Words: Biochemical markers, Early detection of osteoporosis, Postmenopausal women, Western region of Nepal

INTRODUCTION

Osteoporosis is defined as the reduced bone mass per unit volume of normal mineralized bone that leads to fractures, even with minor trauma. Osteoporotic fractures are a common cause of morbidity and mortality in adult men and women. This silently increasing metabolic bone disease is extensively prevalent in developing countries like Nepal. Osteoporosis is a growing health issue and an economic setback. The prevention of osteoporosis requires more focus than the mere detection of the X-ray changes and the BMD scan changes. All over the world, the prevalence of osteoporosis remains to be almost static or increasing in different areas due to the less attention which is being paid to it, as compared to other burning issues and also because of the lack of awareness among the women, especially among the post-menopausal ones. Bone metabolism is a dynamic and constant process which maintains an equilibrium between the resorption of the old and injured bone which is initiated by the osteoclasts and the creation of new bone under the effect of the osteoblasts. Osteoporosis can be present without any symptoms for years, because it doesn't lead to any symptom until the fractures occur. Furthermore, some osteoporotic fractures may escape recognition for years if they do not show any symptoms. After 40 years of age, the bone resorption exceeds the bone formation and the bone density decreases over the years, which in turn may pave the way for osteoporosis [1]. The prevalence of osteoporosis increases with age for all the bone sites, and by the WHO definition, up to 70% of the women who are more than the 80 years of encompass osteoporosis. Investigations like duel energy X-ray de-absorptiometry (DEXA) are being used for screening women who would be more vulnerable to osteoporosis, hence enabling us to manage them in a better way, but biochemical markers are a cheaper and easier way of detecting early osteopaenia and osteoporosis prone women, especially in places where the DEXA scan facilities are not so readily available [2]. The detection of the change in the bone mineral density (BMD) helps in managing the disease progression and it supposedly offers a chance for intervention to reduce the fracture risk for the individual [2]. The purpose of this study was to achieve an easy and early detection of osteoporosis in post-menopausal women, while detecting the more vulnerable premenopausal women also.

MATERIALS AND METHODS

This was a hospital based, comparative study which was carried out in the Department of Orthopedics of Manipal Teaching Hospital, Pokhara, Nepal, between 31st December 2009 and 31st July 2011.

The variables which were collected were age (years), years after menopause (years), BMI, total serum calcium (mmol/l), ionized calcium (mmol/l), phosphorus (mmol/l), total protein (g/dl),albumin (g/dl) and ALP (units/L). The approval for the study was obtained from the institutional research ethical committee. The estimations of serum and ionized calcium were done by colourimetric methods [3,4]. The estimation of serum inorganic phosphate and was done by the Direct method [5]. Total proteins were determined by the Biuret method [6]. Albumin was measured by the BCG method [7]. The estimation of alkaline phosphatase was done by the kinetic enzymatic method [8]. The height and weight of all the participants were noted and their body mass index (BMI) was calculated by using the formula: BMI = weight (kg)/height^2 (m). All these laboratory parameters were analyzed by using human reagent kits and a semi auto analyzer (Human, Germany). Their analysis was done by using descriptive statistics and by the testing of the hypothesis. Random urine samples were collected for estimating the urinary hydroxyproline by an isotope-dilution procedure in which the final step was automatic amino acid analysis [9]. The data was analyzed by using Excel 2003, R 2.8.0, the Statistical Package for the Social Sciences (SPSS) for Windows, version 16.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows version. The Chi-square test was used to examine the association between the different variables. The Z-test was used to compare the significant differences between two variables. A p-value of <0.05 (two-tailed) was used to establish the statistical significance.

Inclusion criteria: The study group comprised of post-menopausal women who were in the age group of 45-78 years and pre-menopausal women who were in the age group of 24-47 years.

Exclusion criteria: Smokers, alcoholics and women who were on oral contraceptive pills who received Hormone Replacement Therapy (HRT) and any other medication that could influence the bone turnover were excluded from our study.

RESULTS

Of the 612 subjects, 306 were pre-menopausal and rest of the 306 were postmenopausal women. The post-menopausal women were further categorized into early (132) and late (174) post menopausal women. For all the subjects, the mean values and the p value was calculated with all variables which were taken into our study.

Parameters	Pre-menopausal (306)	Post-menopausal (306)	p-value
Age (years)	32.02 ± 6.5	61.28 ± 7.95	0.0001**
Years after menopause (years)	–	10.53 ± 7.19	–
BMI (kg/m^2)	24.77 ± 2.19	24.77 ± 1.76	0.995
Total serum calcium (mmol/L)	2.22 ± 0.20	2.05 ± 0.11	0.0001**
Ionized calcium (mmol/L)	1.22 ± 0.97	1.16 ± 0.17	0.287
Phosphorus (mmol/L)	1.32 ± 0.17	1.26 ± 0.19	0.03**
Total protein (g/dl)	6.83 ± 0.65	6.93 ± 0.96	0.0001**
Albumin (g/dl)	3.67 ± 0.23	3.89 ± 0.33	0.0001**
Urinary hydroxyproline (mg/ g Cr)	10.17 ± 2.59	26.73 ± 14.56	0.0001**
ALP (units/L)	211.16 ± 37.35	226.44 ± 44.36	0.0001**

[Table/Fig-1]: Comparison of biochemical markers in pre (n=306) and post (n=306) menopausal women: (Statistics expressed as mean+/- SD)
** Statistically significant p<0.05.

[Table/Fig-1] illustrates that there was no significant difference in the mean values of the BMI of the premenopausal (24.77± SD2.19) and those of the post menopausal women [(24.77± SD1.76) p value (0.99)]. The mean values of serum calcium were moderately reduced in the postmenopausal women (2.05± SD0.11) as compared to those in the premenopausal women (2.22 ± SD0.20) and this was found to be statistically significant (p value 0.001). The mean values of ionized calcium were quite similar in both the groups and this was found to be statistically insignificant. Further, there was a mild increase in the mean values of serum phosphorous in the postmenopausal (1.26±SD0.19) women as compared to those in the premenopausal women (1.32±SD0.17). The values of total protein and serum albumin did not show much variation in both the groups. The mean values of ALP were found to be elevated in the post-menopausal (226.44±SD44.36) women as compared to those in the pre-menopausal (211.16±SD37.35). The values of urinary hydroxyproline were raised significantly in the postmenopausal women (26.73+/-14.56) as compared to those in the premenopausal women (10.17+/-2.59), which is found to be statistically very significant on analysis (p value<0.05).

[Table/Fig-2] depicts that there was no significant difference in the mean values of the BMI of the early postmenopausal (24.65± SD1.67) women as compared to those in the late post menopausal women [(24.83± SD1.80) p value (0.63)]. The mean values of serum calcium were less the early (2.06± SD0.09) postmenopausal women and more in the late (2.15± SD0.12) postmenopausal women and this was found to be statistically significant (p value <0.05). On further analysis, the levels of ionized calcium were found to be significantly raised in the late (1.39 ±SD1.16) post-menopausal women as compared to those in the early post menopausal women (1.07± SD0.10). There was no apparent difference in the mean values of serum phosphorous in the early (1.27± SD0.18) and late (1.26±0.19) postmenopausal women (p value 0.63 i.e insignificant). The mean values of ALP were found to be elevated in the early (248.0±SD35.14) postmenopausal women as compared to those in the late postmenopausal (228.0±SD45.84) women and this was found to be statistically significant (p<0.05). The mean values of urinary hydroxyproline were more in the early postmenopausal women (23.07±11.38) than in the late (19.06± 8.73) postmenopausal women.

Parameter	Early (132)	Late (174)	p value
Age (years)	52.53 ± 3.12	65.07 ± 6.22	0.0001**
Years after menopause (years)	2.53 ± 1.44	13.85 ± 5.85	0.0001**
BMI (kg/m^2)	24.65 ± 1.67	24.83 ± 1.80	0.63
Total serum calcium (mmol/L)	2.06 ± 0.09	2.15 ± 0.12	0.0001**
Ionized calcium (mmol/L)	1.07 ± 0.10	1.39 ± 1.16	0.0001**
Phosphorus (mmol/L)	1.27 ± 0.18	1.26 ± 0.19	0.63
Total protein (g/dl)	6.42 ± 0.85	7.15 ± 7.12	0.0001**
Urinary hydroxyproline (mg/ g Cr)	23.07 ± 11.38	19.06 ± 8.73	0.0001**
ALP (units/L)	248.0 ± 35.14	228.0 ± 45.84	0.0001**

[Table/Fig-2]: Comparison of the bone turnover markers between early (n=132) and late (n=174) post menopausal periods in the postmenopausal women in this study.
**Statistically significant p<0.05

DISCUSSION

Osteoporotic fractures are a frequent cause of morbidity and mortality in adult men and women. Osteoporosis can exist without any symptoms for a long time. After the age of 40, the bone resorption exceeds the bone formation and the bone density reduces over the years, which in turn may pave the way for osteoporosis. It restricts one's daily activities and routine work, thus leading to an economic burden too. Measurements of the bone biochemical markers are increasingly being used to evaluate the rates of bone formation and resorption, especially in cases of osteoporosis. Biochemical parameters give an overview of the elevated rate of the bone turnover, which depicts a low bone mass. Also, the biochemical markers can predict as to whose bone loss was high as compared to the normal values (>3% to 5% per year). The present study revealed that there was a reduction in the serum calcium levels in postmenopausal women, thus indicating a need for calcium supplementation for them. Alkaline phosphatase (ALP) is a marker for bone metabolism. Serum alkaline phosphatase has several dimeric isoforms that come from different tissues like the liver, bone, intestine, spleen, kidney, and the placenta [10]. In adults with normal liver functions, about 50% of the total ALP activity is contributed by the liver and 50% is contributed by the bone [10]. Changes in the bone-specific ALP can lag by more than a few weeks. Subsequent to the start of the anti-resorptive therapy, the suppression is evaluated by assessing the resorption markers, as the coupling process gets normalized. In this study, the total ALP levels were considerably raised in the postmenopausal women (226.44±44.36) as compared to those in the premenopausal women (211.16±37.35). The ALP levels were elevated in the early (248.0±35.14) postmenopausal women as compared to those in the late (248.0±48.84) postmenopausal women [Table/Fig-2] [10]. The ionized calcium levels were considerably reduced in the early post menopausal women as compared to those in the late postmenopausal women [Table/Fig-2] [10] ·This indicated that the bone mass continued to fall with age, but at a slower rate than during the early post menopausal period, thus indicating that this marker evaluation proved to be a timely initiative in detecting the target women in the postmenopausal age group for the supplementation of calcium. The calcium salts in the bone exist in the collagen fibrils, 14 % of which is mainly hydroxyproline which is excreted in urine and therefore it can prove to be a useful marker [10,11]. The oestrogen shortage at menopause raises the rate of the bone remodeling, which leads to an elevated bone turnover and the osteoblast receptors stop functioning efficiently due to the lack of hormones, which can be seen as a significant rise in the mean values of the markers of resorption from pre-menopause to post-menopause [10]. In a study which was conducted by Indumati et al (2005), a similar result was found and all the markers like ALP and urinary hydroxyproline were found to be raised in the postmenopausal women. The bone turnover markers have been proved to be the most efficient indicators of the fracture risks in women who had a low bone mass [12,13]. According to a lot of studies which were conducted regarding the initiation of the treatment for osteoporosis, the increased bone turnover markers were found to have an upper hand, as the BMD changes were found to appear later [14]. Appreciable changes in the bone markers could be seen in three to seven months after the initiation of the therapy [14]. There is still more scope for improving the diagnostic accuracy and for confirming the results by the regular follow up of the women with abnormal biochemical markers, to detect those who could develop osteoporosis later in life, hence reinforcing our research work. Hopefully, in future, there will be a follow up study and a subsequent issue of the same research if the required resources will be available.

CONCLUSION

The bone turnover markers are a better way for the early detection of the high risk women and of those in the early phases of osteoporosis when the X-ray and DEXA scan changes are not prominent. These women can be started on vitamin D and calcium supplementations, especially when they are not receiving these via any other sources (like HRT).

REFERENCES

[1] Pi YZ, Wu XP, Liu SP, Luo XH, Cao XZ, Xie H et al. Age-related changes in the bone biochemical markers and their relationship with the bone mineral density in normal Chinese women. *J Bone Miner Metab*. 2006; 24(5):380-5.

[2] Krueger D, Vallarta-Ast N, Checovich M, Gemar D, Binkley N. BMD measurement and precision: A comparison of the GE Lunar Prodigy and the iDXA densitometers. *J Clin Densitom*. 2011;26(3):538-45.

[3] Ripoll JP. Colorimetric determination of the calcium in serum by using methylthymol blue. *Clin Chim Acta*.1976; 72(1):133-9.

[4] Ijaz A, Mehmood T, Qureshi AH, Anwar M, Dilawar M, Hussainl, et al. Estimation of ionized calcium, total calcium and albumin corrected calcium for the diagnosis of hypercalcaemia of malignancy. *J Coll Physicians Surg Pak*. 2006; 16(1):49-52.

[5] Daly JA, Ertingshausen G. A direct method for determining the inorganic phosphate content in serum by using "CentrifiChem". *Clin Chem*.1972 ;18(3):263-5.

[6] Weichselbaum TE. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am J Clin Pathol* 1946; 10:40-9.

[7] Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin by using bromcresol green. *Clin Chim Acta* 1971; 31(1):87-96.

[8] Moss DW. Alkaline phosphatase isoenzymes. Clin Chem. 1982; 28(10): 2007-16.

[9] Adams E, Ramaswamy S, Lamon M. The 3-hydroxyproline content of normal urine. *J Clin Invest* 1978 June; 61(6): 1482–1487. doi: 10.1172/JCI109068.

[10] Indumati.V, Patil VS, Jailkhani R. A hospital based preliminary study on osteoporosis in postmenopausal women. *Indian J Clin Biochem*, 2007; 22(2): 96-100.

[11] Simsek B, Karacaer O, Karaca I. Urine products of bone breakdown as the markers of bone resorption and the clinical usefulness of urinary hydroxyproline: an overview. *Chin Med J (Engl)*. 2004; 117(2):291-5.

[12] Riggs BL, Melton LJ 3rd, O'Fallon WM. Drug therapy for vertebral fractures in osteoporosis: evidence that decreases in the bone turnover and increases in the bone mass both determine the antifracture efficacy. *Bone* 1996; 18: S197-S201.

[13] Vasikaran S D, Glendenning P, Morris H A. The role of the biochemical markers of the bone turnover in the osteoporosis management in the clinical practice. *Clin Biochem Rev*. 2006 August; 27(3): 119–121.

[14] Seibel MJ, Lang M, Geilenkeuser WJ. Interlaboratory variation of the biochemical markers of the bone turnover. *Clin Chem* 2001; 47: 1443-50.

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