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Use of Bone Turnover Markers and Minerals as Indicators of Low Bone Mineral Density in Premenopausal and Postmenopausal Women for Early Detection of Osteoporosis

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ABSTRACT

Morbidity and mortality associated with osteoporosis continues to be high in India primarily due to late diagnosis. The aim of this study was to evaluate the difference in the levels of the bone turnover markers, oestrogen, macrominerals and trace elements in premenopausal & postmenopausal women, in order to assess whether one or more of these markers can be used as indicators for early detection of osteoporosis which can develop in later life. 350 women between 30–65 years were classified into normal, osteopenic and osteoporotic group based on bone mineral density (BMD) and their serum samples were analysed for oestrogen, osteocalcin, telopeptide-c, calcium, phosphorus, magnesium, copper, iron and zinc levels. Both in premenopausal and postmenopausal groups the oestrogen, telopeptide-c, calcium, phosphorus, magnesium and copper levels correlated well with the status of BMD and served as an early warning against development of future osteoporosis. While levels of iron and zinc showed significant alteration only with severe decline in BMD and were not suitable for early warning of future osteoporosis. It is important to monitor not just postmenopausal but even older premenopausal women for bone mineral density. However, instead of measuring bone mineral density alone, oestrogen, telopeptide-c and minerals- calcium, phosphorus, magnesium and copper should also be estimated which can provide us with an early warning of the possibility of future development of osteoporosis.

Key words: *Bone mineral density, Bone turnover markers, Osteopenia, Osteoporosis*

INTRODUCTION

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 occurring spontaneously or as a result of minor trauma. 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.[1]

In females with the onset of menopause, rapid bone loss occurs which is believed to be greatest in the early postmenopausal years.[2] The pathogenesis of postmenopausal osteoporosis involves the interplay of many factors- Nutritional, Environmental & Genetic factors. Several trace elements are essential in bone metabolism.[3] The risk of nutritional disturbances, particularly trace elements and vitamin deficiencies is high during menopause. Ovulatory changes that amplify as women progress through perimenopause are associated with bone loss despite normal estradiol levels and regular menstrual cycles.The lack of estrogen in menopause accelerates bone loss.

Loss of bone tissue can be estimated by measuring bone mineral density (BMD), but it is unable to provide direct information on bone metabolism and also the changes in BMD appear late and are relatively irreversible at this stage.[4] Biochemical measures may have an advantage over measuring BMD during early stages of bone loss. Combined biochemical and BMD screening may provide better prediction of future fracture risk than BMD alone.[5]

Biochemical markers of bone turnover have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone disease as they reflect the whole body rates of bone resorption (Resorption markers) and bone formation (Formation markers).[6] 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 BMD at specific skeletal sites.[7]

It is now firmly established that low bone mass (osteopenia) is a significant risk factor for osteoporotic fractures later in life.[8] Early detection of future osteoporosis is therefore important in premenopausal women for its timely intervention. Studies on bone loss in menopause are numerous in comparison to those on premenopausal women. While BMD measurement has always been used for this purpose, additional measurement of bone turnover markers has gained in importance for more effective monitoring. But there are very few Indian studies regarding the biochemical markers which indicate bone turnover in our setup.

The present study intends to evaluate the difference in the levels of the bone turnover markers, oestrogen, macrominerals and trace elements in pre & postmenopausal women, in order to assess whether one or more of these markers can be used as indicators for early detection of osteoporosis which can develop in later life.

MATERIALS AND METHODS

This was a hospital based cross-sectional study conducted on women aged 30-65 years of middle socioeconomic status of urban Kolar region of Bhopal visiting various outpatient departments of the J.K. Hospital as an attendant. WHO estimates of 33% for prevalence of osteoporosis among the females in India was taken as reference for arriving at the projected sample size. Assuming a prevalence of 33% and absolute precision of 5% with 95% confidence, the required sample size was estimated to be 350. Age group of 30-65 years was selected to cover both premenopausal and postmenopausal groups. The approvals of the Institutional Ethical Committee were obtained prior to conducting the research. Subjects were included in the study after obtaining their informed consent in local vernacular.

Demographic details on educational level, income, occupation, diet, exercising schedule; and medical, obstetrical, menstrual, and drug history were collected using a structured questionnaire. In addition, information on past non traumatic fracture, family history of fracture and osteoporosis was also obtained. To ensure capturing of variation in BMD only on account of age, BMI and menopausal status, and not due to other factors, uniformity in subject profile was ensured by selecting women from middle socioeconomic status, exposed to similar levels of exercising with similar dietary pattern.

Women in pregnancy, lactating, or in postpartum period less than 12 months, carrying any disease or receiving treatment that could affect BMD; receiving/having received any treatment for osteoporosis; having a secondary cause for osteoporosis; suffering from chronic diseases affecting bone; having endocrinopathies; undergoing current or had past treatment with glucocorticosteroids/thyroid hormones/hormone replacement therapy and those using oral contraceptive pills were excluded from the studies.

Women in sample, were categorized into two groups- premenopausal and postmenopausal. Menopausal criteria considered was absence of menses for at least 12 months. Subjects were screened for Bone Mineral Density at calcaneum heel by means of Quantitative Ultrasound. Observed BMD values were used as T-scores to categorize subjects as normal (T-score $\geq$ -1.0), osteopenic (T-score between -1.0 and -2.5) or osteoporotic (T-score $\leq$ -2.5).

Eight milliliters of blood was drawn from each subject and collected by venepuncture using plastic disposable syringes under aseptic measures. The samples were analyzed for osteocalcin, telopeptide-c, oestrogen, calcium, phosphorus, magnesium, copper, iron and zinc. Serum osteocalcin, telopeptide-c and oestrogen were measured by ELISA kits while macrominerals and trace elements were determined by using spectrophotometric kits.

The data obtained was analyzed using SPSS 16 software. The statistical test used was students t-test and anova. The difference between the subjects was considered significant if the P value was less than 0.05.

RESULTS

Mean values of oestrogen, bone turnover markers and minerals were compared in premenopausal and postmenopausal women and their mean difference was found to be significant (P<.001) for all the parameters **(Table I).** Based on the BMD these women were further classified into five subgroups- premenopausal normal BMD, premenopausal low BMD, postmenopausal normal BMD, postmenopausal osteopenic BMD and postmenopausal osteoporotic BMD. **Table II** gives mean values of oestrogen, bone turnover markers and minerals in various subgroups. **Table III & Table IV** gives comparision of means in various subgroups of premenopausal and postmenopausal women.

Oestrogen was found to be significantly lower in women with low BMD in both the groups. However, in the postmenopausal group, level of oestrogen of osteopenic women was lower than the normal group but least in the osteoporotic group.

Osteocalcin levels were not found to be significantly different in women with low BMD in both the groups. In the postmenopausal group, level of osteocalcin did not differ in the subgroups of women who were normal, osteopenic or osteoporotic. Osteocalcin levels of postmenopausal women were however significantly higher than premenopausal women.

Comparing the two groups, telopeptide C levels in the postmenopausal group was significantly higher than the premenopausal group. Telopeptide C was found to be significantly higher in women with low BMD in both the groups. However, in the postmenopausal group, level of telopeptide C of osteopenic women was higher than the normal group but highest in the osteoporotic group.

Comparing the two groups, the levels of calcium, phosphorus, magnesium and copper in the postmenopausal group were significantly lower than in the premenopausal group. Their levels were found to be significantly lower in women with low BMD in both the groups. However, in the postmenopausal group, levels of these minerals in osteopenic women were lower than the normal group but least in the osteoporotic group. Comparing the two groups, the levels of iron and zinc in the postmenopausal group was significantly higher than in the premenopausal group. Their levels were found to be significantly higher in women with low BMD in both the groups. In the postmenopausal group, levels of these minerals were significantly higher only in the osteoporotic women but not in the osteopenic group.

DISCUSSION

In our study oestrogen levels correlated well with the status of BMD and served an early warning even in premenopausal women against development of future osteoporosis. These findings are consistent with the role of oestrogen in maintaining bone strength. Reduction in bone mineral density occurs when there is an imbalance between the creation of new bone and removal of old bone. A decline in estrogen has been shown

to play a major role in this decreased bone mass during the onset of menopause, especially because it has a variety of protective effects on bone marrow and bone cells. [9]

Telopeptide C levels correlated well with the status of BMD and served an early warning even in premenopausal women against development of future osteoporosis. These findings are consistent with the role of telopeptide C as a marker of bone resorption. [10] Although Osteocalcin levels of postmenopausal women were higher than the premenopausal women but they did not correlate with the status of BMD. Osteocalcin levels unexpectedly correlated positively, rather than negatively with those of telopeptide C.[11] These findings are in contrast to the known role of osteocalcin as a marker of bone formation rather than bone resorption. However, as the method detects not just intact osteocalcin but also the cleaved osteocalcin fragments released from bone matrix during bone resorption, it explains the paradoxical positive correlation with telopeptide C. [12] Further studies are needed to explore the role of osteocalcin in diagnosis of postmenopausal osteoporosis and to establish methods to detect only intact osteocalcin and not the degraded fragments.

Since postmenopausal females are exposed to greater risk of serum biochemical changes as compared to the premenopausal females, this leads to an increased risk of osteoporosis after menopause. Our study reported low calcium levels in postmenopausal women reflecting low skeletal mass.[13] Serum calcium levels are lower in postmenopausal women than in pre-menopausal women.[14] Declining ovarian function at menopause is accompanied by reduction in bone mass and altered calcium metabolism.[7] Oestrogen deficiency may induce calcium loss due to decreased intestinal calcium absorption and decreased renal calcium conservation.[15]

Serum osteocalcin levels in postmenopausal osteoporotic women were significantly higher than in premenopausal non-osteoporotic women. Osteocalcin has a high affinity for calcium and exhibits a compact-calcium dependent α-helical conformation, in which the Gla residues binds and promote absorption to hydroxyapatite in the bone matrix, in this way mineralization of bone takes place. In osteoporotic women, deficiency of calcium may lead to lowering of formation of hydroxyapatite crystals. Thus, in the state of decreased rate of bone mineralization, free osteocalcin may be available for circulation in the blood. This may explain the increased concentration of osteocalcin in the serum of osteoporotic postmenopausal women.[16]

Levels of calcium, phosphorus, magnesium and copper correlated well with the status of BMD and served an early warning even in premenopausal women against development of future osteoporosis. These findings are consistent with their roles in bone turnover and metabolism. Levels of these minerals are decreased in post-menopausal women and are believed to relate to disorders of bone metabolism.[17-19] Levels of iron and zinc showed significant alteration only with severity of decline in bone mineral density and were not suitable for early warning of future osteoporosis.[19] Although estrogen decreases by 90%, a concurrent but inverse change occurs in iron levels during menopausal transition.[20]

CONCLUSION

It is important to monitor not just postmenopausal but even older premenopausal women for bone mineral density. However, instead of measuring bone mineral density alone, oestrogen, telopeptide-c and minerals-calcium, phosphorus, magnesium and copper should also be estimated which can provide us with an early warning of the possibility of future development of osteoporosis.

Table 1: Comparision of means in premenopausal and postmenopausal women.

Parameters	Mean (SD)		Mean Difference (95% CI)	T-Score	P value
	Premenopausal (n=180)	Postmenopausal (n=170)			
Oestrogen	208.9 (75.2)	24.5(34.0)	184.4 (172.0,196.8)	29.2	<.001
Osteocalcin	9.0 (1.0)	9.8(1.7)	0.8 (0.5, 1.1)	5.5	<.001
Telopeptide-C	.270 (0.099)	.490(0.135)	0.218 (0.194, 0.243)	17.3	<.001
Calcium	9.0 (0.8)	8.5(0.5)	0.5 (0.3, 0.6)	6.7	<.001
Phosphorus	3.6 (0.6)	2.9(0.4)	0.4 (0.3, 0.5)	6.6	<.001
Magnesium	1.9 (0.3)	1.8(0.2)	0.2 (0.1, 0.2)	6.1	<.001
Copper	96.5 (19.5)	84.3(10.9)	12.2 (8.8, 15.6)	7.1	<.001
Iron	78.2 (18.8)	136.7(19.7)	58.5 (54.4,62.6)	28.3	<.001
Zinc	78.8 (7.3)	99.9(7.8)	21.0 (19.5,22.7)	26.0	<.001

Table II : Mean levels in various subgroups made on the basis of BMD.

Parameters	Mean (SD)				
	Premenopausal		Postmenopausal		
	Group a (n=88)	Group b (n=92)	Group c (n=39)	Group d (n=43)	Group e (n=88)
Oestrogen	223.59 (71.5)	195.1 (76.5)	41.7 (59.0)	37.3 (18.4)	10.5 (1.3)

Osteocalcin	9.2 (1.2)	9.4 (0.9)	9.5 (1.2)	9.4 (1.3)	10.0 (2.0)
Telopeptide-C	.201 (0.038)	.338 (.092)	.322 (0.060)	.435 (.069)	.590 (.087)
Calcium	9.5 (0.8)	8.5 (0.4)	9.3 (0.5)	8.5 (0.3)	8.2 (0.2)
Phosphorus	3.8 (0.6)	2.9 (0.3)	3.6 (0.4)	2.9 (0.3)	2.6 (0.3)
Magnesium	2.2 (0.3)	1.8 (0.1)	2.1 (0.2)	1.8 (0.1)	1.6 (0.1)
Copper	109.3 (20.4)	84.4 (6.9)	97.0 (16.8)	80.8 (4.1)	80.4 (3.2)
Iron	65.0 (9.0)	90.9 (17.1)	127.4 (14.4)	130.6 (21.8)	143.9 (18.2)
Zinc	73.7 (3.6)	83.8 (6.6)	96.4 (6.9)	97.4 (7.8)	102.8 (7.2)
a= premenopausal normal BMD; b= premenopausal low BMD; c= postmenopausal normal BMD; d= postmenopausal osteopenic BMD; e= postmenopausal osteoporotic BMD					

Table III : Comparison of means of various subgroups with subgroup a (students t-test).

	Group comparisons (P values)			
Parameters	**b vs. a**	**c vs. a**	**d vs. a**	**e vs. a**
Oestrogen	<.05	<.001	<.001	<.001
Osteocalcin	NS	NS	NS	NS
Telopeptide-C	<.001	<.001	<.001	<.001
Calcium	<.001	NS	<.001	<.001
Phosphorus	<.001	NS	<.001	<.001
Magnesium	<.001	NS	<.001	<.001
Copper	<.001	<.01	<.001	<.001
Iron	<.001	<.001	<.001	<.001
Zinc	<.001	<.001	<.001	<.001
a= premenopausal normal BMD; b= premenopausal low BMD; c= postmenopausal normal BMD; d= postmenopausal osteopenic BMD; e= postmenopausal osteoporotic BMD				

Table IV : Comparison of means in various subgroups of postmenopausal women (anova).

Parameters	F score	Multigroup comparisons		
		Pvalues		
		d vs. c	e vs. c	d vs. e
Oestrogen	15.9	NS	<.001	<.001
Osteocalcin	2.4	NS	NS	NS
Telopeptide-C	133.9	<.001	<.001	<.001
Calcium	133.9	<.001	<.001	<.001
Phosphorus	143.2	<.001	<.001	<.001
Magnesium	107.4	<.001	<.001	<.001
Copper	54.8	<.001	<.001	NS
Iron	14.9	NS	<.001	<.001
Zinc	15.1	NS	<.001	<.001
c= normal BMD; d= osteopenic BMD; e= osteoporotic BMD				

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