

Original Research Article

Evaluation of bone mineral density and its correlation with homocysteine and other biochemical bone markers in postmenopausal women

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ABSTRACT

Background: Osteoporosis is more common in post-menopausal women. Early detection of bone loss by bone mineral density helps to confirm the diagnosis of osteoporosis and assesses the future risk of osteoporotic fractures. Recent studies have revealed the association between increased plasma concentrations of homocysteine (Hcy), and reduced bone mineral density. Nevertheless, inconsistencies persist in the literature. Thus, the need for this study arose to investigate the possible relationship between serum Hcy status and bone mineral density on a group of postmenopausal women. The objective of the study was to assess bone mineral density (BMD) in postmenopausal women and to correlate the same with biochemical bone markers like homocysteine, serum alkaline phosphatase (ALP), calcium and phosphorous levels.

Methods: One hundred (100) postmenopausal women were recruited to enter this cross-sectional study. Out of which 86 postmenopausal females, were grouped into osteopenic and osteoporotic based on low t –scores. Bone mineral density (BMD) was measured by dual-energy x-ray absorptiometry (DEXA) and serum Hcy, serum ALP, calcium and phosphorus levels were estimated. The relationship of Hcy with BMD and other biochemical markers was estimated using Pearson's correlation.

Results: Serum Hcy levels were significantly higher in osteoporotic women when compared to other BMD groups, and were inversely correlated with BMD. No statistical difference was seen with other biochemical bone markers like calcium, Phosphorus and ALP.

Conclusions: This study shows that Hcy status is associated with BMD in osteoporotic postmenopausal women. BMD evaluation in postmenopausal women with high Hcy levels may have prognostic and therapeutic potentials, which needs to be explored through further Prospective studies.

Keywords: Homocysteine, BMD, Postmenopausal women

INTRODUCTION

India is the second largest emerging economy and second most populated country in the world.¹ Currently, approximately 10% of India's population (more than 100 million) is aged over 50 years. Based on current patterns

of growth, India's population is expected to grow by 16% to reach 1.4 billion by 2025. Those above the age of 50 years will constitute 22% of this population. With estimates showing that approximately 80% of the urban Indian population is vitamin D deficient and hip fractures occur about a decade earlier than in Western nations.^{2,3}

Osteoporosis is a major concern for this ageing population as it is one of the leading cause and risk factor for fractures.⁴

In the 2009 International Osteoporosis Foundation (IOF) Asian Audit, expert groups estimated that the number of osteoporosis patients in India was approximately 26 million in 2003. In 2013, sources estimate that 50 million people in India are either osteoporotic (T-score below -2.5) or have low bone mass, i.e., osteopenic (T-score between -1.0 and -2.5).⁵ Globally, women have 30-40% risk of osteoporosis during their life time.⁴

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. It is now widely accepted that the accelerated rate of bone loss seen after the menopause is mainly due to an uncoupling in bone turnover and an increase in bone resorption due to hormonal action of estrogen.⁶

Clinically, menopause is said to have occurred when menstruation has ceased for twelve months. 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. Studies have shown that both menopause and aging are associated with an accelerated loss of bone mass.⁷

Early detection of bone loss by measurement of bone mineral density helps confirm the diagnosis of osteoporosis and assesses the future risk of osteoporotic fractures so that timely therapy can be instituted. Dual energy x-ray absorptiometry (DEXA) is the gold standard to assess bone mineral density. It has been suggested that combined biochemical markers and BMD screening might be a useful predictor of future fractures than BMD alone.⁴

Biochemical markers reflect small changes in bone turnover of the entire skeleton in a shorter time frame compared with absorptiometry method, and also capture bone properties independent of BMD measurements. These markers have been suggested to reflect postmenopausal high bone turnover and hence could be useful in separating women into fast and slow bone losers.⁸

Recent studies have reported important association between increased plasma concentrations of homocysteine (Hcy), a sulfur containing amino acid, reduced BMD and increased risk of bone fractures. Hyperhomocysteinemia (HHcy) is being considered as a new independent risk factor for osteoporosis and fractures, particularly in the geriatric population.⁹

While some studies show a significant association between Hcy and BMD, other studies negate any significant association. There is lack of information

regarding the risk factors of osteoporosis and paucity of studies regarding the correlation between BMD and other biochemical bone markers in postmenopausal osteoporosis in developing countries. Thus, the present study has been planned to evaluate some important bone markers and their correlation with BMD in postmenopausal women.

Objectives of the study

- To assess bone mineral density (BMD) in postmenopausal women.
- To correlate BMD with biochemical bone markers like homocysteine, alkaline phosphatase, calcium and phosphorous levels in postmenopausal women.

METHODS

The present cross sectional study was carried out from March 2017 to February 2018. The study was conducted in the Department of Orthopaedics, Adichunchanagiri institute of Medical Sciences, Bellur. The study population consisted of 100 postmenopausal females, selected based on convenient sampling technique from general population on the basis of inclusion and exclusion criteria. The Research protocol was approved by the institutional ethics committee.

Inclusion criteria

Healthy postmenopausal women in the age group of 40-70 years who were willing to participate in the study, who have attained menopause for 3 to 5 years and have not started taking hormone replacement therapy, have not used calcium supplementation or any other medications known to affect bone metabolism.

Exclusion criteria

Women with a) secondary osteoporosis; b) bone tuberculosis; c) chronic debilitating diseases of kidney and liver; d) hormonal disorders; e) history of smoking and alcohol consumption; f) on medication with calcium, corticosteroids, anticonvulsant and heparin were excluded.

Procedure

Bone mineral density (BMD) assessment

After obtaining informed consent, BMD assessment was done by DEXA scan of upper end femur and lumbar spine. The subjects were graded as osteopenic and osteoporotic on the basis of t-scores, according to World Health Organization (WHO) classification system.

Estimation of biochemical parameters

About 5 ml of venous blood from all subjects was collected aseptically in fasting state on the same day of

BMD measurements. Serum was separated immediately by centrifugation and kept at -20°C until analysis was carried out. Homocysteine, calcium and phosphorous were measured by appropriate spectrophotometric methods using fully automated biochemistry analyser. Serum alkaline phosphatase was measured by kinetic method recommended by International Federation of Clinical Chemistry.

Statistical analysis

The results for continuous measurements are presented as mean \pm SD. Student's *t* test (two tailed, independent) was used to determine the significance of study parameters on a continuous scale between two groups. The Pearson correlation coefficient was computed to correlate BMD with biochemical bone markers. Correlation of serum markers with other variables like age, time duration since menopause, BMI was also calculated by Pearson's Correlation coefficient method. Instat statistical software was used for statistical analysis. A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Out of 100 postmenopausal females who were selected based on the inclusion and exclusion criteria, 86 subjects were graded as osteopenic (n=43) and osteoporotic (n=43) based on the t-scores of BMD. The mean ages of the women in osteopenic and osteoporotic groups were 57 years and 53 years respectively. The mean serum Calcium levels were reduced compared to normal reference range in both the groups (Table 1) (8.6-10.2 mg/dl). Phosphorous and alkaline phosphatase levels were within normal limits (Phos= 3-4.5 mg/dl) (ALP=52-147 U/L). However there was no statistically significant difference of calcium, phosphorous and alkaline phosphatase levels between the two groups. Hyperhomocysteinemia (HHCy) was observed in both the groups (Normal Hcy Levels= 5–15 $\mu\text{mol/L}$).

Hcy (Table 1, Figure 1) and BMD (Table 1) showed statistically significant difference when both the groups were compared ($p<0.05$).

Table 1: Estimated mean values of age, BMD and biochemical parameters.

Parameters	Osteoporotic group	Osteopenic group	t-value	P value
Age (yrs)	56.74 \pm 10.61	53.02 \pm 7.8	1.852	0.0675
Calcium (mg/dl)	8.35 \pm 0.95	8.51 \pm 0.87	0.8145	0.4177
Phosphorous(mg/dl)	3.68 \pm 0.83	3.37 \pm 0.66	1.915	0.0588
BMD	-3.319 \pm 1.086	-1.14 \pm 1.73	6.986	<0.0001*
Hcy ($\mu\text{mol/L}$)	21.26 \pm 8.29	17.85 \pm 5.93	2.194	0.031*
ALP (U/L)	89.88 \pm 25.61	87.46 \pm 28.0	0.4179	0.6771

BMD= Bone mineral density, Hcy- Homocysteine, ALP = Alkaline Phosphatase $p<0.05$ –significant.

Table 2: Correlation matrix of BMD with other variables (r value).

		Age	ALP	PHOS	Ca	Hcy	BMDt _l
Age	Pearson correlation	1	-0.060	0.132	-0.081	0.271	-0.236
	Sig. (2-tailed)		0.704	0.397	0.607	0.079	0.128
	N	43	43	43	43	43	43
ALP	Pearson correlation	-0.060	1	0.017	-0.242	-0.420**	0.103
	Sig. (2-tailed)	0.704		0.916	0.118	0.005	0.510
	N	43	43	43	43	43	43
PHOS	Pearson correlation	0.132	0.017	1	-0.332*	-0.235	-0.155
	Sig. (2-tailed)	0.397	0.916		0.030	0.129	0.321
	N	43	43	43	43	43	43
Ca	Pearson correlation	-0.081	-0.242	-0.332*	1	0.176	0.093
	Sig. (2-tailed)	0.607	0.118	0.030		0.259	0.555
	N	43	43	43	43	43	43
Hcy	Pearson correlation	0.271	-0.420**	-0.235	0.176	1	-0.353*
	Sig. (2-tailed)	0.079	0.005	0.129	0.259		0.020
	N	43	43	43	43	43	43
BMDt _l	Pearson correlation	-0.236	0.103	-0.155	0.093	-0.353*	1
	Sig. (2-tailed)	0.128	0.510	0.321	0.555	0.020	
	N	43	43	43	43	43	43

** . Correlation is significant at the 0.01 level (2-tailed); * . Correlation is significant at the 0.05 level (2-tailed).

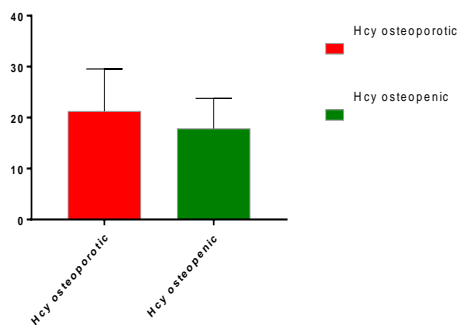


Figure 1: Homocysteine levels among osteopenic and osteoporotic postmenopausal women.

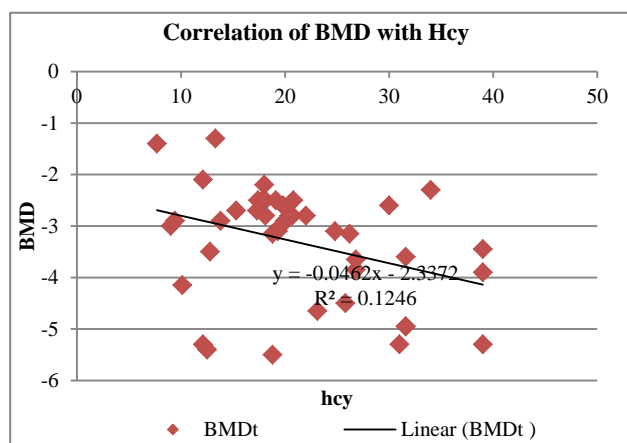


Figure 2: Correlation of BMD with Hcy levels.

When the Hcy levels were correlated with BMD, a statistically significant difference was obtained. Although the osteopenic group had an increased Hcy level and a reduced BMD, there was a much more higher value of Hcy and a further fall in BMD among the osteoporotic group. The scatter plot (Figure 2) shows that the maximum response in the study population fell in the range of 19-22 $\mu\text{mol/L}$ for Hcy and -2.5 to -3.5 SD of BMD. Hence there was a negative correlation between Hcy levels and BMD in this study population (Table 2).

DISCUSSION

The present study showed statistically significant difference in homocysteine levels between osteopenic and osteoporotic postmenopausal women. In addition to the above finding homocysteine also showed statistically significant negative correlation with BMD. Though previous studies have been conducted on the association of Hcy in postmenopausal women data is still inconclusive. While some studies show significant negative correlation of Hcy with BMD, some studies do not show such correlation.¹⁰⁻¹² Present study did not show any difference in calcium, phosphorous and alkaline phosphatase between different BMD groups which is in accordance with different studies by Massé.¹³ Though there is a slight negative correlation of calcium with age

it is not statistically significant, which is in concordance with the study by Onyeukwu et al.¹⁴

High serum homocysteine levels (Hyperhomocysteinemia HHcy) has been implicated as a risk factor for osteoporosis and osteoporotic fractures.⁹ Classical homocystinuria an inborn error of amino acid metabolism caused by cystathionine- β -synthase (CBS) deficiency, manifests as a distinctive spondylo-epimeta-physeal dysplasia, accelerated skeletal growth, osteopenia, elongated appendicular skeleton and flattening of the vertebral bodies.¹³

Several mechanisms have been proposed to link HHcy with the pathogenesis of osteoporosis. Homocysteine has been found to interfere with collagen cross-links. In vitro studies indicate that hyperhomocysteinemia may inhibit the activity of lysyl oxidase (an enzyme involved in cross-linking of collagen) and thereby stimulate osteoclast activity leading to bone resorption. Interference in cross-link formation would cause an altered bone matrix, resulting in more fragile bones.¹⁵

Other mechanisms which have been proposed are reducing osteogenesis by inducing apoptosis in human bone marrow stromal cells, stimulation of osteoclast formation, bone blood flow reduction and changes in bone biomechanical properties, altered gene expression with reduced methylation capacity, oxidative damage of trabecular structures etc.¹⁰

The World Health Organization (WHO) defines osteopenia as the phase before osteoporosis; Present study shows HHcy (elevated Hcy levels) even in osteopenic patients, may indicate a causal relationship of Hcy in osteoporosis.

The present study is in accordance with the study by Bahtiri et al, Bozkurt et al have found that plasma Hcy levels were increased in osteoporosis in a cross-sectional analysis of Turkish postmenopausal women, Zhu et al, also found high Hcy was associated with increased hip bone loss in elderly women.^{10,11,16}

Our study also showed significant negative correlation of Hcy with BMD as in studies by Bahtiri et al, Bucciarelli et al and Baines et al which showed similar findings.^{10,17,18}

Studies by Van Meurs et al, didn't find any association of Hcy and BMD and results from a study on Italian postmenopausal women observed no direct relation between levels of Hcy and BMD.^{19,20} Haliloglu et al also showed that serum Hcy and vitamin B12 levels were not associated with BMD in postmenopausal women.²¹ In addition, a croatian study also showed that Hcy and vitamin B12 levels were not related to BMD regardless of the measurement site.²²

Homocysteine metabolism is closely related to Vit B12, Folate and Vit B6 metabolism, infact hyperhomocysteinemia is seen in vitamin B12 and folate deficiency. Contrasting findings have been observed in relation to vitamin levels and BMD and Hcy. A meta-analysis by Zhang, Tao and Wu showed increase in Hcy and vit 12 but not folate.²³ However a study by Cagnacci et al, showed correlation of BMD with folate but not vit B12.²⁴ Contradicting findings are seen even in reduction of Hcy levels with vitamin B12 and folate supplementation. These findings may indicate that homocysteine may have independent causal association in postmenopausal women.

There are convincing evidences showing HHCy is associated with an increased risk of cardio vascular disease (CVD). Farhat et al, have opined that common pathogenetic factors and epidemiological evidence supports a link between the two diseases osteoporosis and CVD.²⁵ Low bone mineral density (BMD) has been related to increased cardiovascular mortality and cardiovascular morbidity. Hence there is a possibility that HHCy in postmenopausal women may be a pathogenic factor linking osteoporosis with cardiovascular disease. Further studies with large sample size, prospective and multicentric studies are required to prove this association, which may have prognostic and novel therapeutic implications.

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