Original article

Vitamin D, Serum Calcium and Bone Mineral Density in pre and post menopausal women- a pilot study

Dr Lavanya Y, Dr Srikanth S, Satya Chowdary M

Name of the Institute/college: Dr .PSIMS &RF, Chinnavutapalli-521286.A.P India

Corresponding author: Dr Lavanya Yamini

Abstract:

Introduction: Osteoporosis is a major health problem which poses a huge challenge in developing nations like India due to demographic transition and ageing population. .WHO has predicted Asians to be largely affected by osteoporosis by the year 2050. 30 million women in India suffer from osteoporosis, of which 50% are postmenopausal.

Materials and Methods: The study was a cross sectional study conducted in a tertiary care hospital. Thirty women in the age group around 20-40 years (pre menopausal i.e., up to menopause) and thirty two menopausal women 0-5 and 5-10 years after reaching menopause were included in this study.1, 25-$(OH)_2$ Vitamin D was estimated by LUMAX 4100 using Chemiluminiscence method, Serum Calcium by ARSENOZO method using Randox Dytona Auto Analyzer and Bone mineral density using Bone Densitometer Hologic Company.

Observations and Results: We observed a statistically highly significant reduction in vitamin D and serum calcium in post menopausal women when compared to premenopausal women ($p < 0.01$).Also 100% of postmenopausal women had low BMD. Time Duration after menopause also has a significant effect on vitamin D and serum calcium levels along with BMD.

Conclusion: Osteoporosis is an important cause of morbidity and mortality in women. Vitamin D status plays an important role in mineralisation of the skeleton at all ages. Adequate calcium intake along with vitamin D is necessary to maintain the peak bone mass achieved by an individual.

Keywords: Bone mineral density, serum calcium, vitamin D

Introduction:

Osteoporosis is a major health problem which poses a huge challenge in developing nations like India due to demographic transition and ageing population. This is widely prevalent in India. Sixty-one millions in India were reported to be affected by osteoporosis and hip fractures are common in osteoporosis [1, 2]. It was recently reported that Indians have lower bone density than their western and European counterparts [3] and bone mineral density values in Indian population were 15% lower than the western population [4-6].

Extracellular calcium ion concentration is determined by the interaction of calcium absorption from the intestine, renal excretion of calcium, bone uptake and release of calcium, each of which is regulated by parathyroid hormone, vitamin D and calcitonin. Osteocalcin is now considered as an important marker for bone turnover. Bone mass increases during childhood and adolescence, peaks in the third or fourth decade of life, remains stable for some years and declines progressively thereafter, with a sharp acceleration of bone loss during the five to ten years after menopause, ranging from less than 1% to more than 5% per year [7-10].WHO has predicted Asians to be largely affected by osteoporosis by the year 2050 [11]. 30 million women in India suffer from osteoporosis, of which 50% are postmenopausal [12, 13]. Bone turnover increases to high levels and oestrogen deficiency may induce calcium loss by indirect effects on extra skeletal calcium homeostasis.

Bone loss may start before menopause. Serum follicle-stimulating hormone levels (FSH) raise prior to menopause[14-16] and bone turn over markers activity appears to correspond with this raise in FSH [17]. Vitamin D status plays an important role in mineralisation of the skeleton at all ages. Normal bone metabolism depends on the presence of appropriate repletion of vitamin D. Vitamin D insufficiency has been shown to have adverse effects on calcium metabolism, osteoblastic activity, matrix ossification, bone mineral density (BMD), and bone remodelling [18].

Bone Mineral density is the major determinant of the risk for osteoporotic fracture and is preferred to diagnose osteoporosis. It is measured by Dual Energy X-Ray Absorptiometry (DEXA) which is now the gold standard to detect osteoporosis. The most common way of interpreting BMD is to adopt the WHO definition for osteoporosis, based on BMD T-Score. BMD T-Score measures how a subject's BMD value varies to those of a typical young normal subject, expressed in terms of the standard deviation (SD) of young, normal subjects.

Bone mineral density (BMD) declines in women with the onset of menopause associated with increased bone resorption due to reduced estrogen levels. Serum parathyroid hormone (PTH) increases with age and serum 25-hydroxy vitamin D declines with age. Therefore PTH, vitamin D may contribute to bone loss in women.

Vitamin D insufficiency is associated with bone loss and fragile bone fracture. The identification of vulnerable population is important in clinical practice and public health because vitamin D deficiency is readily amenable to dietary vitamin D and calcium supplementation. However, there are several studies on vitamin D status and BMD status in postmenopausal females, but not many studies have been done on vitamin D status among both reproductive and postmenopausal females and associated risk factors. In the present study we undertook the task of assessing vitamin D and BMD among both pre and post menopausal women.

Material and Methods:

The study was a cross sectional study conducted in a tertiary care hospital. Thirty women in the age group around 20-40 years (pre menopausal i.e., up to menopause) and thirty two menopausal women 0-5 and 5-10 years after reaching menopause who were attending the orthopaedic / gynaecology/ medicine OPD of this tertiary care hospital were included in this study. Based on duration of menopause, post menopausal women were divided into two sub groups i.e., group A -0-5 years after menopause; Group B – 5-10 years after menopause.

Inclusion Criteria: Women in the age group 20-40 years of age till menopause and menopausal women 0-5 and 5-10 years after reaching menopause; Women not taking 1,25-(OH)2 Vitamin D3 and calcium supplements for last 6months were included in the study.

Exclusion Criteria : Pregnant women ,Women with known hepatic or renal diseases, Women with known overt diseases of bone and mineral metabolism, Women with known disorders of parathyroid ,Women with known malabsorption syndromes or gastric banding surgeries, Women on drugs like anticonvulsants, thiazide diuretics, steroids, Bisphosphonates, oestrogen or progesterone .

Blood samples for serum 1, 25-$(OH)_2$ Vitamin D3, Calcium were collected from the subjects by venipuncture observing all safety aseptic precautions. The sample was centrifuged and cell free supernatant was aliquoted and stored.

The following data was obtained from all the subjects regarding Age ,Religion ,Place ,Socioeconomic status ,Type of work , Duration of sun exposure – hours/day, Diet history ,Drug

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history – anticonvulsants/steroids/vitamin or calcium/ Bisphosphonates /OCP ;Medical history – chronic liver diseases and Hepatic failure /Nephrotic syndrome and Renal failure / parathyroid disorders /Bone or mineral metabolic disorders like Paget's disease, Rickets, hypocalcemia/ Gastrointestinal malabsorption ;Anthropometric measurements – height, weight, BMI were recorded.

1, 25-$(OH)_2$ Vitamin D was estimated by LUMAX 4100 using Chemiluminiscence method. There has been a long debate on the cut-off points for vitamin D status. The Institute of Medicine considers inadequate if 25(OH) vitamin D levels are <50 nmol/L (<20 ng/mL). However, many consider inadequate/insufficient if levels are <75 nmol/L (30 ng/mL). For the present analysis, the cut off points of Vitamin D were: vit D Deficiency < 12 ng/mL; vit D Insufficiency 12-30 ng/mL) and vit D Sufficient/normal > 30 ng/mL.

Serum Calcium was estimated by ARSENOZO method using Randox Dytona Auto Analyzer considering 8.4-11mg/dl as normal reference interval.

Bone mineral density was measured using Bone Densitometer Hologic Company. Bone mineral density was measured in right calcaneum with bone densitometer.BMD values were measured in terms of T-score.

T-score: It is the difference between the individual patients bone mineral density and the mean results obtained in young adult population expressed in units of young population standard deviation. World Health Organization definition of osteoporosis (T-score at or below -2.5 SD), osteopenia (T-score between -1 and greater than-2.5 SD) and normal (T-score at or above -1 SD) was used.

The study was approved by Institutional Ethical committee.

Statistical analysis: Analysis was done using Mann-Whitney U test and Chi square test to compare the mean values along with SD regarding Vitamin D, serum calcium and T scores of BMD among pre and post menopausal women. The latest version of SPSS software will be used for statistics.

Observation and Results:

Parameter	Group	n	Minimum	Maximum	Mean	Median	SD	P-value
S.VitD3 ng/ml	Pre menopause	30	7.9	38	25.09	26	8.79	<0.01 HS
	Post menopause	32	6.5	28	12.29	10.7	5.57	
S.calcium mg/dl	Pre menopause	30	5.1	11.8	9.74	10.4	1.98	<0.01 HS
	Post menopause	32	5.4	12	7.77	7.45	1.76	
BMD T score	Pre menopause	30	-0.9	-3.18	-1.63	-1.36	0.75	<0.01 HS
	Post menopause	32	-1.1	-5.15	-2.76	-3.025	0.72	

Table 1: Comparison of serum vitaminD3, serum calcium and BMD T score between Pre and Post menopausal women. (Test applied: Mann-whitney U)

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Descriptive statistics of age						
Group	**n**	**Minimum**	**Maximum**	**Mean**	**Median**	**SD**
Pre menopause	30	22	46	36	36	7
Post menopause	32	51	79	62	61	7

Table2: Descriptive statistics of Age

Group	**Vit-D ng/ml**			**Total**	**P-value**
	Deficiency	**Insufficiency**	**Sufficiency**		
Pre menopause	4	14	12	30	<0.01 HS
	13.30%	46.70%	40.00%	100.00%	
Post menopause	24	8	0	32	
	75.00%	25.00%	0.00%	100.00%	
Group	**S. Calcium mg/dl**			**Total**	**P-value**
	Hypocalcemia	**Normal**	**Hypercalcemia**		
Pre menopause	8	12	10	30	<0.01 HS
	26.70%	40.00%	33.30%	100.00%	
Post menopause	26	3	3	32	
	81.30%	9.40%	9.40%	100.00%	

Table 3: Grading of Study population based on vitamin D and serum calcium status (Test applied: Chi-square)

The mean serum Vitamin D3, serum calcium and T score values of Bone Mineral density (BMD) levels in pre menopausal group were 25.09 ± 8.79 ng/ml; 9.74 ± 1.98 and -1.63 ± 0.75 respectively.

The mean serum Vitamin D3, serum calcium and T score values of Bone Mineral density (BMD) levels in post menopausal group were 12.29 ± 5.57 ng/ml; 7.77 ± 1.76 and -2.76 ± 0.72 respectively. The mean serum vit D3, serum calcium and T scores of BMD in Group A post menopausal women were 12.90 ± 6.08 ng/ml,7.95 ± 1.97mg/dl and -2.62 ± 0.64 respectively. The mean serum vit D3, serum calcium and T scores of BMD in Group B post menopausal women were 10.95±4.2 ng/ml,7.37 ± 1.16mg/dl and -3.047 ± 0.83 respectively.

Based on T scores of BMD in pre menopausal women (n=30), we observed osteoporosis in 6 subjects (20%); osteopenia in 21 subjects (70%) and normal bone density in 3 subjects (10%). In postmenopausal women (n=32), osteoporosis was observed in 21 subjects (65.6%); osteopenia in 11 subjects (34.4%) and a normal bone density in none of the subjects.

In premenopausal women, we observed Vitamin D deficiency in 4 subjects (13.30%); Vitamin D insufficiency in 14 subjects (46.70%) and Vitamin D sufficiency in 12 subjects (40%).Similarly, hypocalcemia was seen in 8 subjects(26.70%) ;normal serum calcium in 12 subjects(40%) and hypercalcemia in 10 subjects (33.3%).

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In postmenopausal women(n=32), we observed Vitamin d deficiency in 24 subjects (75%); Vitamin D insufficiency in 8 subjects (25%) and Vitamin D sufficiency in none of the subjects .Similarly, hypocalcemia was seen in 26 subjects(81.30%) ;normal serum calcium in 3 subjects(9.4%) and hypercalcemia in 3 subjects (9.4%).

Discussion:

From the results of our study we observed that 40% of the premenopausal women were vitamin D sufficient and the remaining 60% showed decrease in vitamin D levels (46.70% were vitamin D insufficient and 13.30 % were vitamin D deficient).Among the post menopausal women, 100% of the women showed decrease in vitamin D (25% vitamin D Insufficiency and 75 % vitamin D deficiency). The results of our study correlate with the study of Harinarayan et al [19] and Goswami et al [1]. The low levels of vitamin D in elderly women may be due to inadequate exposure to sunlight and/or poor diet. In addition, aging decreases the skin's capacity to produce vitamin D as reported by MacLaughlin J, Holick MF [20]. Also another cause of decrease in vitamin D may be decrease in the hydroxylation of vitamin D and responsiveness of the intestinal mucosa to circulating vitamin D levels in elderly individuals which was reported by Heaney RP [21]. Vitamin D deficiency leads to poor calcium absorption, high serum PTH concentrations and accelerated bone loss (Collins D, Jasnani C) [22]. It has also been reported that deficiency can lead to loss of muscle strength and increases the risk of fracture. Prevalence of hypovitaminosis D in post-menopausal women was found to be 47% in Thailand, 49% in Malaysia, 90% in Japan, and 92% in South Korea [23].

We observed a statistically highly significant reduction in vitamin D and serum calcium in post menopausal women when compared to premenopausal women ($p < 0.01$). We observed a significant reduction in vitamin D and serum calcium levels in Group B when compared with post menopausal women of group A. Time Duration after menopause also has a significant effect on vitamin D , serum calcium levels and BMD. Vitamin D deficiency and low calcium may cause long standing secondary hyperparathyroidism leading to increased bone turnover causing osteoporotic fractures (Riggs BL) [24].

All individuals undergo loss of bone mass after age 35 years. Skin complexion, poor sun exposure, vegetarian food habits, low milk intake, high phytates in food, and lack of vitamin D food fortification programme may explain the high prevalence of vitamin D deficiency in India despite its sunny climate. Postmenopausal women had significantly lower serum calcium levels than in pre-menopausal women which may be due to decrease in ovarian function after menopause, accompanied by reduction in bone mass and altered calcium metabolism. Oestrogen deficiency may induce calcium loss due to decreased intestinal calcium absorption and decreased renal calcium conservation.

Aging is associated with decreased osteoblast function, decreased calcium absorption, and, in some cases, decreased ability to synthesize Vitamin D (probably at least one cause of decreased calcium absorption). The decrease in osteoblast function is probably responsible for the observed decrease in bone density, and for the decrease in remodeling and repair (and therefore mechanical strength) that occurs with aging.

The high degree of osteoporosis which is statistically significant ($p<0.01$) as observed by mean of BMD T score seen in post menopausal women (-2.76) compared to pre menopausal women (-1.63) may be due to estrogen deficiency after the menopause and age related processes .The findings of our study corroborate with those of

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earlier studies that as age advances, the incidence of low BMD increases [25-27]. Warming L, Hassager C et al [28] studied about BMD with age and observed that bone loss was accelerated by threefold in the immediate postmenopausal period and loss was seen at all sites during the ages of 50–59, after which the rate of bone loss returned to the low rate seen in the premenopausal years.

Chowdhury S et al [29] and Qureshi HJ et al [30] while comparing BMD in pre and post menopausal women in Bangladesh and Pakistan observed from their studies that postmenopausal women have two times more chance of getting low BMD as compared to premenopausal women. The low BMD observed among the post menopausal women in our study is in accordance with the above study.

Our study reports that 90% of premenopausal women had low BMD while the figure was 100% in case of postmenopausal women. Nuti R, Martini G [31] observed that bone mineral density values in both healthy and osteoporotic post menopausal women were significantly lower than premenopausal values and continued to decrease significantly after the onset of menopause.

Limitations of the study:

We did not consider the effect of Body Mass Index (BMI) on BMD. Serum parathyroid hormone (PTH) might have been estimated along with vitamin D. This study is an attempt to address one of the important public health problems which can be controlled if preventive measures are taken at an early stage. Effect of other risk factors could not be investigated as this is a cross-sectional study.

Conclusion:

Osteoporosis is an important cause of morbidity and mortality in women. Screening by bone mineral density measurement is helpful in assessing bone health in women. In our present study the decline in BMD is not only confined to post menopausal women but also found in pre menopausal women which suggest the cause for bone loss and osteoporotic fractures cannot be implied to menopause or estrogen status and age alone. Bone loss has occurred prior to menopause as evident by BMD status. In elucidating the causes for this bone loss, among the several risk factors, assessment of vitamin D status may provide an important clue as decrease in BMD is also associated with decrease in vitamin D in most of our subjects. It is essential to interpret bone mineral density (BMD) with Vitamin D levels.

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Conflicts of Interest: None

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