STUDY OF BONE MINERAL DENSITY AND SERUM VITAMIN D LEVELS IN HEALTHY POSTMENOPAUSAL WOMEN

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ABSTRACT

INTRODUCTION

Vitamin D metabolites participate in the regulation of calcium homoeostasis and bone metabolism. Severe and prolonged vitamin D deficiency causes osteomalacia. Subclinical vitamin D deficiency is common in the elderly and may lead to development of secondary hyperparathyroidism and bone loss, for which reason it has been implicated in the pathogenesis of senile osteoporosis.

AIM

To determine the relationships between serum 25 (OH) D, bone mineral density, and serum calcium and phosphorus in 50 postmenopausal women.

MATERIALS AND METHODS

A population-based cross-sectional study with a total of 50 healthy women aged over 50 years with amenorrhoea for 12 months were included. None of the women were using hormone replacement therapy. Measurements of bone mineral density were performed by dual-energy X-ray absorptiometry (DXA). Analysis of serum levels of 25(OH) D, calcium, phosphate and alkaline phosphatase were done.

RESULTS

There exists an inverse correlation between BMD and 25(OH) D levels ($r = -0.127$, $p>0.05$) and that between 25 (OH) D and phosphate levels among postmenopausal women ($r = -0.167$, $p >0.05$), which is not significant. No significant difference exists in the serum levels of calcium, alkaline phosphatase between vitamin D insufficient & deficient persons ($p >0.05$).

CONCLUSION

Elevated levels of serum 25(OH) D were associated with decreased bone mineral density and increased bone turnover, but it was insignificant.

KEYWORDS

25(OH) D, Bone Mineral Density, Calcium, Phosphate.

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INTRODUCTION: Osteoporosis, the most prevalent metabolic bone disease in developed countries is characterised by the loss of bone mass, microarchitectural disruption of bone tissue that results in increased risk of fragility fractures, which are the main consequences of the disease.[1] Expert groups peg the number of osteoporosis patients in India at approximately 26 million (2003 figures), with the numbers projected to increase to 36 million by

2013.[2] Osteoporotic fractures are more common in the elderly and result in excess morbidity and mortality in this population. Fractures following "minimal trauma", especially of the vertebra, are the most common and earliest manifestations of osteoporosis.[3] Osteoporosis-related fractures are associated with substantial pain, suffering, disability and possibly even death for the affected patients.

For the first 20 years after the cessation of ovarian oestrogen secretion, postmenopausal osteoporosis accounts for a 50% reduction in trabecular bone and 30% loss of cortical bone. Various factors are involved in the aetiology of osteoporosis among Indian women. The non-modifiable factors include sex, advancing age, ethnicity and genetic factors. The modifiable risk factors of osteoporosis include nutritional factors, urbanisation, decreased sun exposure and lesser physical activity, which are detrimental to bone health. Ageing is associated with decreased sun exposure, oral intake and skin activation of Vitamin D, and Vitamin D absorption. All of these factors may contribute to Vitamin D insufficiency, which is required for calcium absorption and bone mineralisation. The low serum Vitamin D is associated with increased PTH secretion, which in turn leads to bone resorption and increased renal calcium excretion.[4]

Vitamin D inadequacy affects 64% of osteoporotic women worldwide.[5] Various factors like latitude, season and time of the day, pigmentation of the skin, ageing and the topical use of a sunscreen influence the amount of synthesis of vitamin D in the skin.[6] Cholecalciferol (Vitamin D3) is synthesized in skin from 7-dehydrocholesterol on exposure to sunlight. This is then hydroxylated in the liver to form 25-hydroxy cholecalciferol which is again hydroxylated in the kidney by 1-α hydroxylase to form 1, 25-dihydroxycholecalciferol, the active form of vitamin D3. Most of the tissues having vitamin D receptors generate 1, 25-dihydroxycholecalciferol in a paracrine fashion from 25-hydroxycholecalciferol. This paracrine effect may be responsible for its role in cell growth regulation, the adaptive and innate immune system functioning, insulin secretion by pancreatic β cells, blood pressure regulation and normal neuromuscular function. The major risk factors for vitamin D inadequacy are high BMI, inadequate vitamin D supplementation and poor self-reported health.[7] The primary age-related change in bone mass occurs when there is an imbalance in bone formation and bone resorption. Instead of a comparable degree of bone formation and resorption, there is decreased bone formation (osteoblastic activity) and increased bone resorption (osteoclastic activity). This shift in bone remodelling usually begins in the third decade of life and continues with ageing.[8] The increased osteoclastic activity is significantly exacerbated by oestrogen loss, especially during menopause in women. After menopause, there is a drastic change in the androgen-to-oestrogen ratio because of the sharp decrease in estradiol levels and slightly reduced testosterone.

Oestrogen deficiency causes both the early and late forms of osteoporosis in postmenopausal women and contributes to the development of osteoporosis in elderly men[9]. It is associated with large increase in bone resorption caused by increased osteoclast numbers (due to enhanced osteoclast formation and reduced osteoclast apoptosis) and by increased osteoclast activity.[10] Oestrogen induces osteoclast apoptosis, a process that was inhibited by antibodies to TGF-ß.[11] Also, oestrogen directly targets RANKL-induced osteoclast differentiation by repressing c-Jun activation and that this response was dependent on the oestrogen receptor.[12] Two features of osteoporosis suggest a role for local factors in pathogenesis. They are systemic hormones that influence the skeleton, including oestrogen and PTH which alter the production of local factors (e.g., cytokines, prostaglandins, and growth factors) and differential bone loss that occurs in different parts of the skeleton.[13] Bone markers are used to assess bone turnover in patients with osteoporosis, because the rate of bone turnover (spontaneous or modified by the therapy) is considered an important determinant of bone fragility in postmenopausal and older women. Elevated markers of bone turnover indicate increased bone turnover but are not diagnostic of osteoporosis. They are useful in monitoring the effects of anti-resorptive therapy. Osteoporosis is a systemic bone disorder typified by low bone mineral density (BMD).

The gold standard for the diagnosis of osteoporosis is by dual energy X-ray absorptiometry (DXA), a specialised X-ray device that precisely quantifies BMD at the spine, femur, and other skeletal sites. DXA scans are noninvasive and comfortable for the patient, with very low radiation requiring only 10 minutes for the entire examination. In the DXA technique, two x-ray energies are used to estimate the area of mineralised tissue and the mineral content is divided by the area, which partially corrects for body size. However, this correction is only partial since DXA is a two-dimensional scanning technique and cannot estimate the depths or posteroanterior length of the bone[14]. Local factors affecting bone density such as weight-bearing, muscle usage and co-existing osteoarthritis may result in differences in BMD categories between bones.

AIM: To estimate the serum 25(OH) D levels and to compare serum levels of 25(OH) D with BMD in patients with osteoporosis.

MATERIALS AND METHODS: The study was cross-sectional in design and it was conducted at Mahatma Gandhi Memorial Government Hospital attached to K.A.P.V. Government Medical College, Trichy after getting approval from the institutional ethics committee. 50 female postmenopausal patients with amenorrhoea for more than 12 months and those who have completed 50 years of age and willing to participate in the study were included. Patients who had hormonal disorders, renal diseases, metabolic bone disorders and who were on immunosuppressive drugs were not selected for the study. Patients with history of surgery, hospitalisation or major medical illness within the past 1 year were excluded from the study. A detailed medical, obstetrical, menstrual and drug history was recorded in a proforma designated for the study. Appropriate tests to exclude secondary causes of osteoporosis were based on the

history and physical findings. Informed consent was obtained from all subjects prior to the study. Blood sample was collected to assess the serum alkaline phosphatase, calcium and phosphorus, 25(OH) D levels and all patients underwent DXA scan. Under aseptic precautions, 5 mL of venous blood sample was collected after an overnight fasting of 12 hours from all subjects. After retraction of the clot, samples were centrifuged at 2000 rpm for 15 minutes for separation of serum. All blood samples were obtained from the subjects under fasting conditions and serum was stored at -80˚C until analysis and had a single thaw at the time of analysis.

Vitamin D nutritional status is best determined by the measurement of 25(OH) D rather than 1, 25(OH) D because 25(OH) D is the main circulating form of Vitamin D and day-to-day variation is less due to its long half-life. Also measurement of 25(OH) D is comparatively easier than the more technically complicated methods for 1, 25(OH) D.[15] Vitamin D levels were classified as sufficient (≥ 30 ng/mL), insufficient (11-29 ng/mL) and deficient (≤10 ng/mL).[16] 25 (OH) D was estimated by fully automated chemiluminescent immunoassay analyser. BMD was measured at the femoral neck on the right side and left side and the lumbar spines L1 - L4. The DXA scan report was given by the radiologist based on the WHO classification of BMD T-score. It classifies the patients as normal: between 1 and -1, osteopenia: -1 to -2.5, osteoporosis: -2.5 or lower.[17] After testing, the patients were informed about the results of their investigations and counselled regarding the risk of osteoporosis.

Serum alkaline phosphatase, calcium and phosphorus levels were measured by fully automated biochemistry analyser. Serum phosphorus and alkaline phosphatase are useful in ruling out hyperparathyroidism and osteomalacia. Anthropometric measurements were taken. Statistical analysis was done using SPSS version 16 software. Analysis included standard descriptive statistics. Two-sample student t-tests were performed to compare normally distributed variables between groups. For comparisons of normally distributed variables between groups, t-test was used. The 'p'-values in the text and tables were two-tailed. The significance limit was $p<0.05$. Correlation between the measured parameters were assessed by calculating Pearson's correlation coefficient.

RESULTS: Table-1 shows the descriptive statistics of the study group as mean±standard deviation. Mean age of study population was 71.24±9.8. Mean vitamin D levels of the study population is 13.98±5.3 ng/mL. The mean levels of serum calcium, phosphorus and alkaline phosphatase are 8.32±0.82 mg/dL, 6.39±1.14 mg/dL and 128.74±29.7 U/L respectively. Table 2 allows for the comparison of serum 25(OH) D levels in the study group (n=50) where serum 25(OH) D levels in Vitamin D insufficient (11- 29 ng/mL) category (n=39) are 15.73±4.37 ng/mL and that in Vitamin D deficient (≤10 ng/mL) category (n=11) are 7.19±2.24 ng/mL. From Table 3, it is seen that there exists an inverse correlation between BMD and 25(OH) D levels (r = - 0.17 and -0.13 in vitamin D insufficient and deficient postmenopausal women respectively, $p>0.05$) and that between 25 (OH) D and phosphate levels among postmenopausal women (r = -0.24 and -0.16 in Vitamin D insufficient and deficient postmenopausal women respectively, $p >0.05$) which is not significant. No significant difference exists in the serum levels of calcium, alkaline phosphatase between vitamin D insufficient & deficient persons ($p >0.05$).

Sl. No.	Parameters	Postmenopausal women (n=50)			
		Min	Max	Mean	Sd
1	Age (years)	55	92	71.24	9.8
2	Calcium (mg/dL)	6	9.8	8.32	0.82
3	Phosphate (mg/dL)	4.6	10.3	6.39	1.14
4	ALP (U/L)	66	194	128.7	29.77
5	25-OH D (ng/mL)	4.2	25.4	13.98	5.3
6	Bone Mineral Density (BMD)	0.25	-5.7	-3.24	1.29

***Table 1: Descriptive Statistics of the Study Group* (n=50)**

Serum	Vit.D Insufficient (n=39)	Vit.D Deficient (n=11)	Student 't' Test p Value
Mean	15.73	7.19	<0.001*
Standard deviation	4.37	2.24	

Table 2: Comparison of Serum 25-OH D Levels in the Study Group (n=50)

* Significant at 0.05 level.

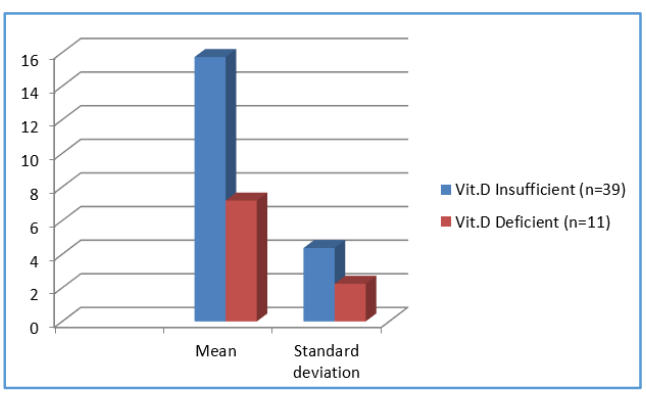

Fig. 1: Comparison of Serum 25-OH D Levels in the Study Group (n=50)

Sl. No	Parameters	Vit.D Insufficient(n=39)		Vit.D Deficient (n=11)	
		Pearson Correlation (r)	p value	Pearson Correlation (r)	p value
1	Calcium (mg/dL)	0.15	>0.05	0.55	>0.05
2	Phosphate (mg/dL)	-0.24		-0.16	
3	ALP (U/L)	0.34		-0.47	
4	Bone Mineral Density(BMD)	-0.17		-0.13	

Table 3: Pearson Correlation Matrix between Serum vit. D and Various Parameters among vit. D Insufficient and vit. D Deficient Postmenopausal Women

DISCUSSION: This cross-sectional study deals with a homogenous group of women with respect to age and menopausal status, as they are included 12 months after their last menstrual bleed. Furthermore, they were screened for diseases known to influence bone metabolism. Such a group therefore seems suitable for studies into relationships between bone mass, dietary factors and biochemical parameters that exist within the normal physiological range.

While evaluating the vitamin D status of the study population, 78% (n=39) of the population had vitamin D insufficiency and 22% (n=11) had deficiency. The decrease in serum 25 (OH) D levels may be attributed to the lifestyle change of people with less outdoor activities with decreased sunlight exposure, thereby reducing the ultraviolet-B (UVB)-induced vitamin D synthesis in the skin.[18] Vitamin D is then transported to the liver and converted to 25-hydroxy vitamin D (25(OH)D) which is the major circulating and storage form of vitamin D.

Vitamin D status is considered as an important determinant of bone health. Vitamin D deficiency in adults induces secondary hyperparathyroidism, which leads to loss of bone matrix and minerals. These factors lead to increased risk of osteoporosis and fractures. At age 50, women have a lifetime risk of about 40%.

One third of women older than 65 years suffer vertebral crush fractures. Vertebral crush fractures can occur acutely resulting in disabling pain and discomfort. Long term complications include immobility and loss of height. Secondary problems include protuberant abdomen, chronic constipation and loss of self-esteem. Peak bone mass is normally attained by 30 years of age and decreases by 30-45 years in both men and women. Exercise and adequate nutrition play important roles in attaining and maintaining skeletal mass.

The amount of bone attained during growth is an important determinant of whether osteoporosis develops later in life. During early adult life, bone formation is coupled to bone resorption so that bone mass remains stable. After 35-40 years of age, bone resorption slightly exceeds bone formation so that approximately 1% of skeletal mass is lost per year. In women, the decrease in sex steroids at menopause (postmenopausal osteoporosis) accelerates bone loss to about 2% per year.[19]

Advanced age, female gender and sex steroid deficiency are prominent risk factors for osteoporosis. Other risk factors include a family history of osteoporosis, alcohol abuse, smoking and chronic diseases. After decreased bone mass is documented by bone mass measurements, the diagnostic workup is directed at determining the cause. Most often the cause is attributed to age ("senile osteoporosis"), postmenopausal osteoporosis or both. But it may be secondary to chronic diseases, drug therapies or other causes. Serious consequences on overall health and well-being is also associated with Vitamin D deficiency.[20]

Our data demonstrated a marginal inverse association between serum 25(OH) D levels and BMD in postmenopausal women which is not significant (p>0.05) similar to Harinarayan CV et al.[21] Based on our results, it may be recommended to take vitamin D supplements when the circulating 25(OH) D level is below 30 ng/mL, especially in postmenopausal women.

LIMITATIONS: Because of the small sample size of the study population and cross-sectional design of the study, no sufficient causality could be demonstrated for the relationship between serum 25(OH) D and other variables. Estimation of serum PTH would have helped better in assessing the disease process of osteoporosis.

CONCLUSION: Vitamin D insufficiency and deficiency is common but a largely ignored health problem in healthy postmenopausal women living in South India. Low serum 25(OH) D levels were found in healthy postmenopausal women. Prevention of osteoporosis is an important goal. Adequate nutrition and exercise during growth allow achievement of optimal bone mass. High prevalence of low serum 25 (OH) D levels suggest that modifiable risk factors such as inadequate vitamin D supplementation and low sun exposure could reasonably be targeted as part of a comprehensive strategy to improve bone health in postmenopausal women. With so much said about Vitamin D deficiency and insufficiency, one must be careful not to overenthusiastically overtreat Vitamin D deficiency without actually monitoring Vitamin D levels and land up with Hypervitaminosis D.

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