



## STUDY ON THE VITAMIN D STATUS IN RELATION TO AGE, BONE MINERAL DENSITY OF SPINE AND FEMUR IN KANPUR (INDIA) FEMALES WITH BACKACHE – A HOSPITAL-BASED STUDY

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### Abstract

Our aim in the present study was to evaluate the association between bone mineral density in lumberspine and femoral neck with serum levels of vitamin D in females of Kanpur, Uttar Pradesh, India, aged between 25 to 65 years. Recent attention to the high prevalence of osteoporosis and its association with low vitamin D levels in adults has raised the importance of vitamin D evaluation. A low level of vitamin D is considered to be one of the most important risk factors for osteoporosis. The present study was a cross sectional study carried out for a period of 1 year. i.e, April 2022 to April 2023. A total of 200 females without any diagnosed chronic disease attending the outpatient department at Rama Medical College Hospital and Research Centre, Mandhana, Kanpur, India, were recruited randomly were included in the study. The serum levels of total Vitamin D were taken to be severely deficient if lower than 20 ng/ml, mild to moderate deficient if it was between 20 and 30 ng/ml and optimum level if it was 31–100 ng/ml. We observed that 6.6%, 17.1%, 26.7% and 20.0% women suffered severe deficiency of Vitamin D among the age groups A (25-35 years old), B (35-45 years old), C (45-55 years old) and D (55-65 years old) respectively. While, 30.0%, 67.14, 69.3 and 72.0% had mild deficiency, whereas, 63.3%, 15.7%, 4.0% and 8.0 of female among the same groups possessed optimal level of Vitamin D. Overall 20% women suffered from severe, 63% had mild to moderate while 17% showed normal levels of Vit D. And these results indicated that the age group C (45-55 years) had a slightly higher percentage of Vit D deficiency. Only 4-8% of women above the age of 45 had normal Vit D levels. The BMI in all groups showed a significant correlation with the mineral deficiency and osteoporosis. Out of the 200 women included in the study 122 suffered from Osteopenia while 78 had normal bone density. The highest incidence of osteopenia was seen in the Group C (55-65 year) i.e. 76% while the lowest observed in group A (25-35 years) which was 30.0% among the respective groups. The females with a higher BMI had lower levels of osteopenia and no subject had indications of osteoporosis across the four groups which is a promising indication of better dietary status with respect to Vitamin D.

**Keywords:** BMD, BMI, Vitamin D, Osteopenia, Osteoporosis, Serum

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## **Introduction**

Vitamin D is a group of fat-soluble secosteroids responsible mainly for increasing intestinal absorption of calcium, magnesium, and phosphate. Vitamin D plays a critical role in bone metabolism and many cellular and immunological processes. Low levels of vitamin D have been associated with various chronic diseases especially rickets in children and osteoporosis in adults especially in women. Osteoporosis is a common bone disorder that is characterized by a reduction in the bone mass and disruption of bone architecture leading to impaired skeletal strength and increased susceptibility to fragility fractures. It is also known by the unique name of "silent disease," or "silent thief" because most of the times patients and their physicians become aware of the condition only after person falls and sustain fracture.

Osteoporosis affects postmenopausal females, particularly the elderly, and is a prevalent and complicated health issue [1]. It is a progressive skeletal disease characterised by decreasing bone mineral density (BMD) and collateral bone microarchitecture destruction, which may therefore compromise skeletal strength and increase fracture risk [2].

According to statistics, more than 50% of persons aged 50 or older have osteoporosis, with postmenopausal females making up over 70% of this group [3]. Additionally, in 2010, more than 900 million women over 50 had osteoporosis. Over 10 million females are expected to suffer negative effects by 2020 due to the lack of efficient disease-prevention techniques [4]. The use of BMD as a substitute measure for the diagnosis of osteopenia and osteoporosis is widely recognised [5]. This further validates the value of BMD as a diagnostic tool for aberrant bone mass and osteoporosis [6]. Clinically, a person with BMD higher than 2.5 SD below the adult mean value is regarded to have acquired osteoporosis. In general, a number of diverse factors have been linked to BMD, including body weight, physical activity, and exercise, as well as nutrients like calcium and vitamin D, alcohol, coffee, and body weight. Body weight and body mass index (BMI) [7,8], among these variables, have been found to have a significant influence on postmenopausal females' BMD [9].

Lean mass (LM) and fat mass (FM) make up the majority of the body weight in healthy persons with FM making up the remaining 16–25% of total body weight [10]. Males and females have

different relationships between LM and BMD, and postmenopausal females have a stronger effect of FM on BMD than premenopausal females do [11]. Body FM has been identified as the most important predictor of BMD in postmenopausal women in few earlier research [12,13]. In contrast, a person's BMI, which is a calculated number based on their weight and height, allows doctors to classify them as underweight, normal weight, overweight, or obese based on how much muscle, fat, and bone they have in their body [14]. Increased BMI has been shown to have a protective effect on bone density, and those who are moderately overweight were found to have elevated BMD values, suggesting that a relationship between BMI and weight gain and BMD may exist [15]. More importantly, it has been shown that a rise in body weight is associated with endocrine changes that can either directly or indirectly have a favourable impact on bone metabolism [16] investigations [17,18] provided evidence linking LM and FM to bone mass. However, a specific BMI value chart to accurately predict osteoporosis and related fracture risk remains to be fully established. Preliminary results suggested that a BMI of 26~28 may confer some protection, whereas a BMI of 22~24 likely indicates an increased risk [19]. Therefore, we carried out the current analysis and sought to clarify whether body weight and BMI have an important impact on the BMD of lumbar vertebra and femoral neck in postmenopausal females. It is essential to target bone health in women by identifying specific risk factors and developing awareness programs. Moreover, failure to attain sufficient peak bone mass in childhood and adolescence and lack of maintenance of peak bone mass for a sufficient period of time during early adulthood increases the risk of osteoporosis.

Therefore, the present study was undertaken to study and analyze the relationship between body mass index (BMI) and weight and bone mineral density (BMD) measured in lumbar spine (L1-L4), femur neck in a population of 200 women at a women attending Rama Medical College Hospital and Research Center, Uttar Pradesh. The aim of our study is to find out Bone mineral density (B.M.D) of proximal femur & lumbar spine in women of different age group (age 25 to 65 years) & correlating it with serum vitamin D.

## **Materials and Methods**

The present study was a observational study carried out in the Department of Biochemistry with collaboration with the Radiology Department

for a period of 1 year i.e, April 2022 to April 2023 at Rama Medical College Hospital and Research Centre (RMCH&RC), Kanpur, U.P. The Ethical clearance was obtained from the Institutional Ethical Committee of RMCH & RC, Mandhana Kanpur.

A total of 200 cases of bone deformity were included in the study. All the samples obtained after a written informed consent.

#### **Inclusion Criteria**

- 1) Patients in the age group of 25 - 65 years.
- 2) Patients ready to participate in the study.

#### **Exclusion Criteria**

- 1) Patients with history of fractures due to minor trauma or osteoporosis.
- 2) Patients with metabolic bone diseases, malignancy, renal failure etc.
- 3) Patients with terminal illness, psychiatric illness and severe dementia.
- 4) Patients under the hormone therapy, consumption of dietary supplements, ovarian surgery.
- 5) Patients using the medications that affect bone mass such as those given to treat convulsion (anti-convulsion), respiratory disease (Glucocorticoid), and thyroid problem.

**Sample collection** – Females with complain of backache with no diagnosed chronic diseases attending the outpatient department of RMCH&RC were studied. Out Patients in the age group 25-65 were chosen for the data collection. These subjects had visited the Rama Hospital for routine checkup and volunteered to participate willingly were included in the study. In this study morphometric measurement of the proximal femur and lumbar spine in the different age group women were carried out by bone mineral density.

#### **Tools of the study**

#### **Formula for BMI**

#### **(c) Body Mass Index**

The BMI was calculated as per the guide lines given by the WHO, as follows:

$$\text{BMI} = \frac{\text{Weight (in kilograms)}}{\text{Height (in meters)}^2}$$

Using the above equation, BMI was calculated until these decimal value.

The study tools included an interview questionnaire, anthropometric measurements and blood biochemical tests.

#### **Anthropometric measurements**

The recording of all the anthropometric measurements were done with minimum clothes without shoes and was conducted on the guidelines issued by the World Health Organization (WHO). The Age was recorded to the nearest completed year. Weight (kg), and height (cm) were selected for anthropometric evaluation as variables for calculating BMI (kilogram/meter<sup>2</sup>).

**Weight:** Weight of the subjects was measured by using a portable electronic weighing machine. The same machine was used in case of all women. The machine was calibrated every day before using it. The scale was adjusted to the zero mark. The patient was made to stand bare-footed on the weighing machine without any support, with the feet flat on weighing machine and arms by the side of the body. The patient was made to look straight with eyes parallel to the floor. Body weight was measured in kilograms and measured to the nearest 100gm.

**Height:** Height was recorded with the standard Stadiometer. The patient was made to stand in bare-foot. It was ensured that the occiput, shoulders, buttocks and heels of the patient touches the flat vertical surface of the wall. The patient was made to look straight ahead with the line of sight parallel to the floor with the hands by the side of body. The highest point (vertex) of the head of the patient was felt and the point was marked with a marker. While measuring, the measurer's eyes were at the same level as that of scale touching the head. Height was measured in centimetres and recorded to the nearest 0.5cm [20].

### BMI classification according to World Health Organization(WHO)

BMI(kg/m <sup>2</sup> )	Classification
<25.00	Normal
≥25.00-<30.00	Pre-obese
≥30.00	Obese

### Bone Mineral Density measurements-

Dual energy X-ray absorptiometry (DXA) scan was done to assess BMD. BMD of proximal femur (F-BMD) and lumbar spine (L-BMD) was measured using **WIPRO GE DPX-NT**. While measuring F-BMD and L-BMD with DXA their mean value was considered as T-score

### BMD classification according to World Health Organization (WHO)

BMD(T-score)	Category
>-1	Normal
<-1to>-2.5	Osteopenia
<-2.5	Osteoporosis

### Bone mineral density measurements

BMD (grams/centimeter square) was determined for the anteroposterior or lumbar spine (L1-L4) and mean of proximal right and left femur (total and sub-regions) by dual-energy X-ray absorptiometry (DXA), according to standard protocol. BMD values were classified according to WHO criteria; a T-score between  $-1$  and  $-2.5$  is indicative of osteopenia, while a T-score  $<-2.5$  reflects osteoporosis and a T-score  $>-1$  is considered normal (WHO, 1994) [21].

**Biochemical assessment of Vitamin D** Blood (4 mL) was withdrawn by a nurse after an overnight fast ( $>12$  h) and transferred immediately into non-heparinized tube. Serum samples were stored at  $-80$  °C until required for analysis. Serum total levels of Vitamin D were measured by radio immune assay using Wallac 1470 Gamma Counter (Wallac Inc., Gaithersburg, MD, USA). The serum levels of total Vitamin D were graded as severely deficient if it was lower than 20 ng/mL, mild to moderate deficient if it was between 20 and 30 ng/mL and optimum level if it was 30–100 ng/mL.

### Statistical analysis

Quantitative data were statistically represented in terms minimum, maximum, mean, standard deviation (SD). Comparison between difference groups in the present study was done using One-way ANOVA Test. Qualitative data were statistically represented in terms number and

percent. Comparison between difference groups in the present study was done using Chi-Square Test. A probability value (p value) less than or equal to (0.001) was considered significant.

All statistical analysis was performed using statistical software SPSS (Statistical Package for Social Science) statistical program version(16.0).

### Results

**Vit D Result** - A total of 200 studies from Kanpur were analysed. In all the studies, the criteria for Vitamin D deficiency were kept below 20 ng/ml. The age group of the participants in the studies included in the article ranged from 25 to 65 years. Samples of the present study were categorized in each variable. According to age; 15.0% were 25-35 years old, 35.0% were 35-45 years old, 37.5% were 45-55 years old and 12.5% were 55-65 years. According to serum total levels of Vitamin D (D2 & D3); 21% showed Severe Vitamin D deficiency (lower than 20 ng/ml), 66% have Mild to moderate Vitamin D deficiency (20–30 ng/ml), while 13% were in the Optimum level (30–100 ng/ml).

Among 200 selected subjects in this study, the ages were ranged from 25 to 65. Serum levels of total vitamin D showed wide range from a Minimum  $6.3 \pm 1.4$  to Maximum  $52.9 \pm 33.7$  ng/ml. BMI was ranged from a lowest of 16.2 to a highest of 36.0 Kg/m<sup>2</sup>. The results indicate that 11.25% of the women under study were Obese and 25.5% were overweight as BMI over 30 is considered obese according to WHO. Minimum, maximum, mean and SD of all variables in this study are listed in Table 2. Those with normal body mass index (BMI) had a higher Vitamin D level compared to the population with higher BMI.

By measuring Bone mineral density in lumbar spine; 39.0 % were normal (with t score bigger than  $-1$ ), 61.0 % showed Osteopenia (with t score between  $-1$  and  $-2.5$ ), while none were recorded with Osteoporosis (with t score lower than  $-2.5$ ) (Table 4).

In the present study it was observed that the maximum number of cases were found in the age group of 45-55 years of age with 75 (37.5%) and least was recorded in the age group of 55-65



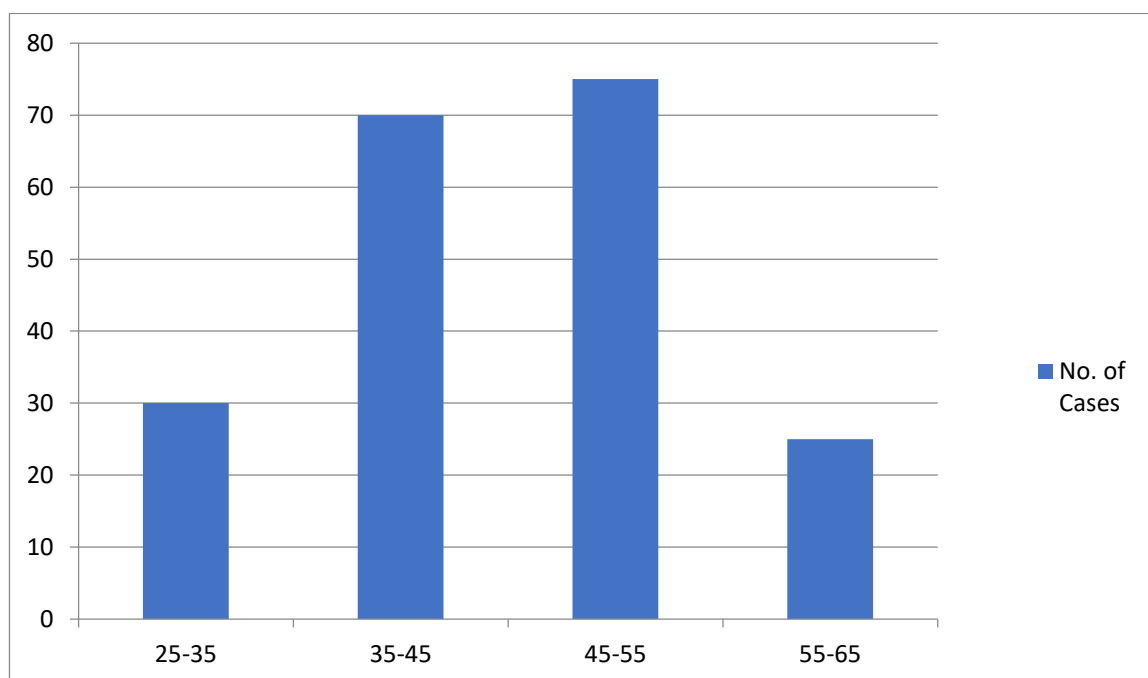
(12.5%) years of age followed by 25-35 (15%) years of age. It was also noted that in the Height parameter, the age group of 75 was observed to be the maximum with the mean± SD of 152.3± 7.1. It was also noted that the weight was observed to be maximum with the gradual increase in the age group. In the current study it was also noted that the BMI was found to be the maximum in the age group of 45-55 years of age [Table 1]. From the table 2 it was noted that the age group of 25 years was the minimum whereas the maximum age group studied was 65 years with the SD of ± 3.01. The minimum Serum total levels of vitamin D (D2 & D3) was 6.3 whereas the maximum was 67. The BMD in Lumbar spine (Normal range  $\geq -1$  to  $\leq -2.5$ ) ranges minimum for 0.854 and the

maximum was 1.137. The minimum BMD in the Femoral neck (BMI) observed was 0.657 and maximum was 1.106. The BMI (Kg/m<sup>2</sup>) recorded minimum of 16.2 and the maximum value observed was 36.

In the present study it was found that in the Serum total levels of vitamin D the maximum number of cases were observed in 126 (63%) of Mild to moderate deficiency (20–30 ng/ml) and 35 (17%) cases were of Optimum level (30–100 ng/ml). In the T- Score BMD of lumbar region and T- Score BMD of Femoral region the maximum 122 (61%) cases were observed of Osteopenia followed by 78 (39%) cases of normal individuals. There was no cases of osteoporosis observed.

**Table 1.** Anthropometric variables in different age group women

Parameters	Group- A (25-35 years) (n = 30)	Group-B (35-45 years) (n= 70)	Group-C (45-55 years) (n = 75)]	Group-D (55-65 years) (n =25)
Age(years) mean± SD	26.2 ± 3.1	35.6 ±2.7	48.35 ± 3.7	56.7± 2.1
Height(cm) mean± SD	153.7± 4.1	159.2± 6.1	152.3± 7.1	163.47± 5.0
Weight(kg) mean± SD	54.9± 8.3	62.7± 7.2	58.07± 10.1	62.3± 7.5
BMI mean± SD	23.46± 1.03	25.08± 2.6	25.24±4.7	23.9±3.8



**Graph No. 1:** Graphical Representation of the Age wise Distribution

**Table 2.** Variables of the study

Variable	N	Minimum	Maximum	Mean	Std. Deviation
Age	200	25	64	42.5	± 3.01
Serum total levels of vitamin D (D2 & D3)	200	6.3	67	42.9	± 0.330
BMD in Lumbar spine (Normal range $\geq -1$ to $\leq -2.5$ )	200	0.854	1.137	0.914	± 0.095
BMD in Femoral Neck	200	0.657	1.106	0.861	± 0.111
BMI ( $\text{Kg/m}^2$ )	200	16.2	36.0	25.25	± 3.55

BMD (Bone mineral density).

BMI (Body mass index).

**\*ICMR Standard cut off for Osteoporosis 0.624, 0.428, and 0.717 gm/cm<sup>2</sup> in hip, fore arm and spine respectively.**

**\*Low BMD was recorded in 59.1% of females [22].**

**Table 3** Distribution of respondents according to age and BMI

Variable	Group	n	%	BMI		
				Normal	Overweight(%)	Obese(%)
Age	25-35 years old	30	15	(22) 74%	(6) 20 %	(2) 7%
	35-45 years old	70	35	(50) 71%	(15) 22%	(5) 7%
	45-55 years old	75	37.5	(43) 57%	(24) 32%	(8) 11%
	55-65 years old	25	12.5	(13) 52%	(7) 28%	(5) 20%

**Table 4** General description of categorical variables

Variable	Category	n (total =200)	%
Serum total levels of vitamin D	Min 6.3 ±1.4 Max 52.9 ±33.7		
	Severe deficiency (lower than 20ng/ml)	39	20
	Mild to moderate deficiency (20–30 ng/ml)	126	63
	Optimum level (30–100 ng/ml)	35	17
T- Score BMD L	Min 0.854 ±0.13 Max 1.137 ±33.7		
	Normal (bigger than $-1$ )	78	39
	Osteopenia ( $-1$ and $-2.5$ )	122	61
	Osteoporosis (lower than $-2.5$ )	0	0
T- Score BMD F	Min 0.657 ±0.14 Max 1.106 ±33.7		
	Normal (bigger than $-1$ )	78	39
	Osteopenia ( $-1$ and $-2.5$ )	122	61
	Osteoporosis (lower than $-2.5$ )	0	0
Age	25-35 years old	30	15
	35-45 years old	70	35
	45-55 years old	75	37.5

55-65 years old

25

12.5

BMD L (Bone mineral density in lumber spine).  
BMD F (Bone mineral density in femoral neck).

**Table 5** Association between each of the variables and age

Variable	Category	Group A 25-35 years old (n=30)		Group B 35-45 years old (n=70)		Group C 45-55 years old (n=75)		Group D 55-65 years old (n=25)		p value*
		n	%	n	%	n	%	n	%	
Serum total levels of vitamin D	Severe deficiency	2	6.6	12	17.1	20	26.7	5	20.0	0.013
	Mild to moderate deficiency	9	30.0	47	67.14	52	69.3	18	72.0	
	Optimum level	19	63.3	11	15.7	3	4.0	2	8.0	
T-score BMD L	Normal	21	70.0	26	37.1	25	33.3	6	24	0.000
	Osteopenia	9	30.0	44	62.9	50	66.6	19	76	
	Osteoporosis	0	0.0	0	0.0	0	0.0	0	0.0	
T-score BMD F	Normal	21	70.0	26	37.1	25	33.3	6	24	0.000
	Osteopenia	9	30.0	44	62.9	50	66.6	19	76	
	Osteoporosis	0	0.0	0	0.0	0	0.0	0	0.0	

BMD L (Bone mineral density in lumber spine).  
BMD F (Bone mineral density in femoral neck).

\* Chi-square test.

\*\* P < 0.001

**Table 6** Association between Vit D levels and Osteoporosis

Subgroup	Category	n	Mean ± SD	P value*
Serum total levels of vitamin D	Severe deficiency	39	82.5±6.5	0.013
	Mild to moderate deficiency	126		
	Optimum level	35		
T-score BMD L	Normal	78	62.0±8.6	0.000
	Osteopenia	122		
	Osteoporosis	0		
T-score BMD F	Normal	78	62.0± 8.6	0.000
	Osteopenia	122		
	Osteoporosis	0		

## Discussion

The most important function of vitamin D, one of the fat-solvent hormones, is the hemostasis of calcium [23]. Numerous symptoms of osteomalacia, including pain, tenderness, muscle weakness, and even trouble walking, can indicate vitamin D deficiency, ranging from generalised musculoskeletal pain to these [24,25]. Our body's persistent low back pain is influenced by low vitamin D levels [25], which can also cause

greater pain sensitivity and reduced neurological and muscle function [26]. Vitamin D deficiency increases the likelihood of inflammatory activity at the vertebral endplates, which lowers pain threshold and causes generalised pain in the muscle and bone, which causes weakening [27]. Few studies assessed the association between vitamin D and intensity of pain, and some pointed out a strong correlation between both the variables [28].

In the present study a total of 200 cases were included in the study out of which the maximum number of cases was observed in the age group of 45-55 years of age followed by 35-45 years of age. This study was similar to the study performed by the other author SentitemsuAo et al., where the maximum number of cases observed was in the age group of 20-35 years followed by 50 years and above [29]. Another study was in support to the present study where according to age 26.7% were 30–39 years old, 40% were 40–49 years old and 33.3% were 50–59 years old.

In the present study the BMI mean± SD was observed minimum with 23.46± 1.03 and the maximum with 25.24±4.7. Similar study was in supported to the present study where the BMI ranged from 32 to 37 that indicates all subjects were obese as BMI over 30 is considered obese according to WHO [29]. In the present study it was found that in the Serum total levels of vitamin D the maximum number of cases were observed in 126 (63%) of Mild to moderate deficiency (20–30 ng/ml) and 35 (17%) cases were of Optimum level (30–100 ng/ml). In the T-Score BMD of lumbar region and T- Score BMD of Femoral region the maximum 122 (61%) cases were observed of Osteopenia followed by 78(39% ) cases of normal individuals. There was no cases of osteoporosis observed. Similar study was performed by the other research investigator where 3 (9.4%) of the younger age group (18-35years) and 7 (35%) of the older age group (≥50 years) were osteopenic among all subjects with normal 25(OH)D levels, whereas the rest had normal BMD levels [29]. In the present study it was also concluded that the Bone loss was observed more at lumbar spine. Lunt *et al* [30] have attributed greater risk of vertebral deformities among women to higher rate of bone loss. This decline in BMD is steeper among women during their transition from fifth to sixth decade, which reflects the impact of menopause on BMD.

The lower bone density among Indians and the earlier on set of osteoporotic fractures among Indians indicate that the cutoffs may need to be revised appropriately for early identification of osteoporosis among Indians. A study on 450 urban healthy women between 25 to 75 years of age that determined the bone mineral density revealed that only 29% had normal T-score[31] Several studies from India have reported that BMD values in Indian women were approximately 5 to 15% lower than those in Caucasian women[32-36]. Such a variation was

also seen among Asian women residing in America[37]. Age-related change in BMD varies with skeletal sites, peak BMD in women was observed between 31 and 40 years of age at the hip and spine [38]. We also observed a higher percentage of women with normal BMD in this age group (70.0 % women in that age group (25-35) and 37.1% normal in the age group 35-45 years which are higher than the other older age groups included in the study.

Studies have reported that menopause occurs at a younger age in women in India compared to Caucasian populations[39]. Studies have also reported low Vitamin D levels in the Indian population[40]. Decrease in serum concentrations of vitamin D would induce reduction in density of cortical bones and may have a supportive role for density of trabecular bones[41].

We found higher levels of serum Vitamin D in the age group 25-35; equal to 63.3 % while lowest percentage of women having normal Vit D was observed ie 4% was in 45-55 age group. The slightly better percentage of normal females (8%) were seen in the highest age group could be due to lesser responsibilities and opportunity to take rest after retirement of completion of family responsibilities, where as the age group 45-55 has sufficient proportion of working ladies or with younger children who demand a lot of care and physical exercise (labour). Singh et al. (2023) have reported a prevalence of Vit D deficiency among the reproductive age group [42] whereas our findings clearly indicate a substantial deficit of this Vitamin in the subjects of post reproductive group much more than the reproductive group average.

## CONCLUSION

In the current study, women with higher BMIs had lower levels of osteopenia, and there were no subjects in any of the four groups who showed signs of osteoporosis, which is an encouraging sign of improved dietary status with regard to calcium and vitamin D as compared to prior decades.

## Conflicts of Interest: None

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