



## A STUDY ON MORPHOMETRY AND BONE MINERAL DENSITY OF PROXIMAL FEMUR AND IT'S CORRELATION WITH SERUM VITAMIN D & SERUM CALCIUM IN PRE-MENOPAUSAL AND POST-MENOPAUSAL WOMEN

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## Abstract

**Background:** Femur is the strongest bone in the body. Morphometric evaluation of proximal femur have predictive role in determining fracture risk. Morphometric parameters of proximal femur are affected by several factors such as age, BMI, calcium, vitamin D and bone mineral density. Thus, the aim of this study was to evaluate femoral morphometry and its relationship with BMD and serum levels of calcium and vitamin D.

**Materials and methods:** This study was conducted in the Department of Anatomy and Department of Orthopedics, Rama Medical College and Research Center, Rama University, Mandhana, Kanpur, UP. Total of 200 healthy women between the age of 25-64 years were included. Details of participants like age, BMI, menstrual status were noted and femoral morphometric parameter like hip axis length (HAL), femoral neck axis length (FAL), femoral neck width and length (FNW, FNL) and femoral neck shaft angle (FNSA) were evaluated. Serum calcium and vitamin D were measured using standard kit-based method and bone mineral density was measured by DEXA.

**Result:** In this study, the level of serum calcium, vitamin D and BMD were significantly high in premenopausal women. 14.4% and 23.3% of premenopausal women and 47.3% and 60.9% of post-menopausal women were vitamin D and calcium deficient respectively. 46.6% and 33.3% of premenopausal women were osteoporotic and osteopenia respectively while the same were 55.4% and 36.4% in postmenopausal women. Proximal femoral morphometry correlated significantly and positively with BMD. The correlations of BMD with calcium and vitamin D were also significantly positive in the study participants.

**Conclusion:** Deficiency of vitamin D and calcium is associated with altered bone structure and mass. Hence, adequate intake of both calcium and vitamin D is needed maintain the normal bone morphometry.

**Keywords:** Calcium, vitamin D, Bone mineral density, osteoporosis

## Introduction

Skeletal components in various populations differ in terms of genetics, environment, nutrition and lifestyle. Since skeletal components and their sizes show racial variations, their morphometric assessment may be used for differentiation between the populations or between the individuals of same population. Further, such evaluations are useful guides to clinicians when

information about fracture risk and surgery are required. Fractures especially of weight bearing bones can be a surgical emergency in health care system because of its complications such as disability and death [1].

Amongst the bones, hip fracture is considered major health problems especially in elderly. Despite of bone strength and density, dimension of femoral head is considered an important risk factor for femoral neck and hip fractures[2]. The other risk factors associated with hip fracture include bone mineral density (BMD), body mass index (BMI), femoral morphometry, amount and direction of force applied to bone, muscle strength, lifestyle, family history etc[3, 4].

Structurally, femur consists of proximal part, shaft and distal part. Proximal part comprises head, neck and trochanters (greater and lesser) [5]. Femoral head supports the weight of the body and this biomechanical property depends on width and length of femoral neck [6]. The mechanical resistance conferred by femoral head is associated with the morphometric parameters such as length of hip axis, length of femoral neck axis, width of femoral neck, width of femoral head, distance between trochanters, and the angle between body and neck of femur. The factors associated with high risk of fracture include greater hip axis length, greater width of femoral neck and greater angle between body and neck of femur [7].

Hip fracture is one of the severe outcomes of osteoporosis. It increases the risk of premature death and mortality rates. According to the World Health Organization (WHO), if BMD of a patient is less than the mean value for a healthy young woman by 2.5 standard deviation, then it provides diagnostic confirmation of osteoporosis [8]. Several factors are associated with reduction in BMD e.g. endocrine dysfunction, medicinal side effects, deficient intake of calcium and vitamin D in diet. Vitamin D, also known as calcitriol, is a seco-steroid that maintains calcium and phosphate homeostasis. Serum levels of vitamin D correlate positively with BMD which means that deficiency of vitamin D reduces BMD and increases the risk of fractures [9]. Calcium, fifth most abundant element in body, has key functions in several physiological process, one of the major functions being mineralization of bone [10].

In India, around 30 million women are affected by osteoporosis, among which about 50% of the cases involve post-menopausal women [11]. Oestrogen deficiency in post-menopausal stage indirectly induces loss of calcium from bone. Normal bone metabolism depends on the blood levels of vitamin D at all ages. Deficiency of vitamin D adversely affects bone health (matrix ossification and osteoblastic activity), BMD and bone remodeling [12]. Besides balanced diet, physical activity and BMI, several reproductive factors are also associated with BMD in both pre and post-menopausal women. These include age of menarche, pregnancy or lactating status. As per previous studies, BMD is high in the adult women who had onset of menarche at an early age (below 12 years) compared to those having delayed menarche. Similarly, BMD is high in post-menopausal women graced motherhood during adolescence stage compared to those who did not in that stage [13].

Since, deficiency of vitamin D is associated with bone loss, bone fragility and fracture especially in the weight bearing bones, it is necessary to identify vulnerable population in clinical practices. Therefore, in this study we aimed to analyse morphometry and BMD of proximal femur and correlate them with serum vitamin D and calcium level among pre and post-menopausal women.

## **Materials and methods**

This cross-sectional study was conducted in the Department of Anatomy and Department of Orthopedics, Rama Medical College and Research Center, Rama university, Mandhana, Kanpur, UP. Total 200 healthy women in the age group of 25 to 64 years and without any bone deformities were included in the study. According to age, they were subdivided into 4 categories viz, 25-34 years, 35-44 years, 45-54 years and 55-64 years.

### ***Inclusion criteria***

- Women of age group 25 to 64 years
- Patients willing to participate in the study
- Patients without bone deformities

### ***Exclusion criteria***

- Patients with history of fractures due to minor trauma or osteoporosis
- Patients with metabolic bone diseases, malignancy, renal failure, thyroid and parathyroid disorders
- Patients with hepatic illness, psychiatric illness, terminal illness and severe dementia
- Patients receiving hormone therapy, dietary supplements and ovarian surgery
- Patients under anticonvulsants, glucocorticoids, diuretics and thyroid medication

After obtaining ethical clearance from the institute, this study was commenced. The anthropometric variables like height and weight were measured. Height was measured using stadiometer while weight was measured using the electronic balance. From height and weight, BMI was calculated as follows:

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{height (m}^2\text{)}}$$

Details of the patients like age, menstrual status, postmenopausal duration, physical activity, dietary habits, any medication etc were noted. Blood sample was collected by vein puncture method following all the precautions. The sample was centrifuged and clear serum was collected. Serum sample was stored at -20°C till analysis.

Serum levels of vitamin D and calcium were analyzed using standard kit-based methods. Bone density measurement was carried out using DEXA (Dual Energy X-ray Absorptiometry) scan. WIPRO GE DPX – NT was used for this purpose. BMD values were expressed in terms of T-

score. T- score is a difference between BMD of individual patients and mean results of young adult population expressed as units of young population standard deviation.

According to WHO, T score  $\leq -2.5$  SD is considered a case of osteoporosis, T score  $\geq -1$  SD is considered normal and T score between -1 to  $> 2.5$  SD is considered osteopenia.

Based on vitamin D levels, the cutoff points established were:

- Vitamin D deficiency:  $< 12$  ng/mL
- Vitamin D insufficiency: 12-30 ng/mL
- Vitamin D sufficiency (normal):  $> 30$  ng/mL

For serum calcium, the cutoff values established were:

- Hypocalcemia:  $< 9$  mg/dL
- Normal: 9-11 mg/dL
- Hypercalcemia:  $> 11$  mg/dL

The morphometric parameters of femur analysed were as follows:

*Hip Axis Length (HAL)in(mm):* Distance from the base of the lateral part of the greater trochanter to the inner pelvic brim.

*Femoral Neck Axis Length(FAL)in(mm):* Length from the base of the lateral part of the greater trochanter to the caput femoris.

*Femoral Neck Width(FNW)in(mm):* Narrowest cross section of the femoral neck.

*Femoral Neck Length(FNL)in(mm):* Distance from the femoral shaft axis to the center of the femoral head along the neck axis.

*Femoral Shaft Width(FSW)in(mm):* Width 3cm below the center of the lesser trochanter.

*Femoral Neck-Shaft Angle(FNSA in degree):* Angle between the femoral neck& shaftofthe femur.

### **Statistical analysis**

It was done using SPSS 25. The comparative analysis was done using students' t-test while correlation was determined by Pearson's correlation coefficient (r). The association of age and menstrual status with BMD, serum calcium and vitamin D was determined with chi square test. P value less than 0.05 indicated statistical significance

## Result

Table 1: Distribution of patients on the basis of age

| Age (years) | Number (N) | Percentage (%) |
|-------------|------------|----------------|
| 25-34       | 38         | 19             |
| 35-44       | 49         | 24.5           |
| 45-54       | 63         | 31.5           |
| 55-64       | 50         | 25             |

Table 2: Distribution of patients on the basis of menstrual status

| Group           | Number (N) | Percentage (%) |
|-----------------|------------|----------------|
| Pre-menopausal  | 90         | 45             |
| Post-menopausal | 110        | 55             |

Table 3: Distribution of post-menopausal women based on duration of menopause

| Duration (years) | Number (N) | Percentage (%) |
|------------------|------------|----------------|
| 0-5              | 25         | 22.7           |
| 5-10             | 37         | 33.6           |
| >10              | 48         | 43.6           |
| Total            | 110        | 100            |

Table 4: Morphometric evaluation of proximal femur on right and left side

| Parameters                                  | Left side<br>(Mean±SD) | Right side<br>(Mean±SD) | P                   |
|---|------------------------|-------------------------|---------------------|
| Hip axis length (HAL in mm)                 | 121.75 ± 9.63          | 118.38 ± 5.41           | 0.632 <sup>NS</sup> |
| Femoral neck axis length (FAL in mm)        | 105.36 ± 3.67          | 97.83 ± 8.32            | 0.115 <sup>NS</sup> |
| Femoral neck length (FNL in mm)             | 27.44 ± 3.21           | 24.39 ± 3.15            | 0.193 <sup>NS</sup> |
| Femoral neck width (FNW in mm)              | 34.81 ± 3.75           | 33.84 ± 5.24            | 0.41 <sup>NS</sup>  |
| Femoral neck - shaft angle (FNSA in degree) | 120.85 ± 2.95          | 120.41 ± 4.06           | 0.683 <sup>NS</sup> |

NS: Non-significant ( $p>0.05$ )

Table 5: Comparison of bone mineral density, calcium and vitamin D between pre and postmenopausal

| Group          | BMD T score | Calcium (mg/dL) | Vitamin D (ng/mL) |
|----------------|-------------|-----------------|-------------------|
| Pre-menopause  | -1.48       | 10.5            | 28                |
| Post menopause | -3.51       | 8.2             | 12.9              |
| p              | 0.001**     | 0.004**         | 0.001**           |

\*\*→P&lt;0.01: Statistical significance

Table 6: Correlation of morphometrical parameters of femur with Age, BMI and BMD

| <b>Parameter</b>                  | <b>Age</b> |                     | <b>BMI</b> |                     | <b>BMD</b> |          |
|-----------------------------------|------------|---------------------|------------|---------------------|------------|----------|
|                                   | <b>r</b>   | <b>P</b>            | <b>r</b>   | <b>p</b>            | <b>r</b>   | <b>p</b> |
| Hip axis length (HAL)             | 0.18       | 0.627 <sup>NS</sup> | - 0.09     | 0.828 <sup>NS</sup> | - 0.621    | 0.001**  |
| Femoral neck axis length (FAL)    | 0.21       | 0.585 <sup>NS</sup> | - 0.17     | 0.652 <sup>NS</sup> | - 0.54     | 0.003**  |
| Femoral neck length (FNL)         | 0.11       | 0.751 <sup>NS</sup> | - 0.15     | 0.758 <sup>NS</sup> | - 0.736    | 0.001**  |
| Femoral neck width (FNW)          | 0.16       | 0.716 <sup>NS</sup> | - 0.08     | 0.789 <sup>NS</sup> | - 0.665    | 0.001**  |
| Femoral neck - shaft angle (FNSA) | 0.03       | 0.882 <sup>NS</sup> | - 0.12     | 0.697 <sup>NS</sup> | - 0.863    | 0.001**  |

\*\*→P<0.01: Statistical significance, NS: Non-significant (p>0.05)

Table 7: Correlation of BMD with serum calcium and vitamin D in pre and post-menopausal women.

| <b>Parameter</b> | <b>Pre-menopausal</b> |                     | <b>Postmenopausal</b> |                     |
|------------------|-----------------------|---------------------|-----------------------|---------------------|
|                  | <b>R</b>              | <b>P</b>            | <b>r</b>              | <b>p</b>            |
| BMD – Ca         | 0.63                  | 0.001 <sup>NS</sup> | 0.52                  | 0.002 <sup>NS</sup> |
| BMD – Vit D      | 0.71                  | 0.001 <sup>NS</sup> | 0.68                  | 0.001 <sup>NS</sup> |

\*\*→P<0.01: Statistical significance, NS: Non-significant (p>0.05)

Table 8: Comparative evaluation of T-score in pre-menopausal and post-menopausal women

| <b>Group</b>            | <b>Normal (N=37)</b> | <b>Osteopenia (70)</b> | <b>Osteoporosis (N=103))</b> |
|-------------------------|----------------------|------------------------|------------------------------|
| Pre-menopausal (N=90)   | 28 (31.1%)           | 30 (33.3)              | 42 (46.6%)                   |
| Post-menopausal (N=110) | 9 (8.1 %)            | 40 (36.4%)             | 61 (55.4%)                   |

Table 9: Association of vitamin D and calcium in pre-menopausal and post-menopausal women

| <b>Vitamin D</b>        |                           |                             |                             |                       |
|-------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------|
| <b>Group</b>            | <b>Sufficiency (N=61)</b> | <b>Insufficiency (N=74)</b> | <b>Deficiency (N=65)</b>    | $\chi^2$ ( <b>p</b> ) |
| Pre-menopausal (N=90)   | 41 (45.5%)                | 36 (40%)                    | 13 (14.4%)                  | 0.005**               |
| Post-menopausal (N=110) | 20 (18.1%)                | 38 (34.5%)                  | 52 (47.3%)                  |                       |
| <b>Calcium</b>          |                           |                             |                             |                       |
| <b>Group</b>            | <b>Normal (N=65)</b>      | <b>Hypocalcemia (N=88)</b>  | <b>Hypercalcemia (N=47)</b> | $\chi^2$ ( <b>p</b> ) |
| Pre-menopausal (N=90)   | 36 (40%)                  | 21 (23.3%)                  | 33 (36.6%)                  | 0.002**               |
| Post-menopausal (N=110) | 29 (26.3%)                | 67 (60.9%)                  | 14 (12.7%)                  |                       |

\*\*→P<0.01: Statistical significance

In this study, among 200 women, maximum number of participants were from 45-54 years age group (31.5%) followed by the age group of 55-64 years (25%) (table 1). 45% of women belonged to pre-menopausal category while 55% of women were in the post-menopausal category (table 2). Among the post-menopausal women, 22.7%, 33.6% & 43.6% of females respectively had post-menopausal duration of 0-5, 5-10 and >10 years (table 3).

In the present study, the morphometric parameters of proximal femur viz. HAL, FAL, FNL, FNW and FNSA were also evaluated in both right and left femur bone. The results were noted as mean  $\pm$  SD and compared with students' t-tests. These parameters of proximal femur did not differ significantly among the right and left sides (table 4).

Bone mineral density in terms of T-score, serum levels of calcium and vitamin D were also compared between pre-menopausal and post-menopausal women. There was significant difference in the mean value of BMD, serum calcium and vitamin D levels. All these parameters were significantly high the pre-menopausal women compared to post-menopausal women (table 5).

The morphometric parameters of proximal femur were also correlated with age, BMI and BMD of the study participants. Significant correlation of BMD with proximal femoral parameters was observed while age and BMI did not show any significant correlation (table 6).

BMD was correlated with serum calcium and vitamin D levels. The correlation was significantly positive in both pre-menopausal and post-menopausal women. The correlation was more significant in case of pre-menopausal women (table 7).

On the basis of T-score, 20%, 52.2% and 27.7% of pre-menopausal women were observed to have normal bone, osteoporosis and osteopenia respectively while in case of post-menopausal women, 8%, 55.4%, and 36.4% of the participants were found to have normal bone, osteoporosis and osteopenia bone respectively (table 8).

14.4% of premenopausal women were vitamin D deficient while it was 47.3% in case of post-menopausal women. Likewise, 23.3% of premenopausal women were hypocalcemic while it was 60.9% in case of post-menopausal women (table 9).

## Discussion

Femur is one of the major parts of human skeleton as it is important of performing routine works and balancing the body. The morphometry of femur is significant determinant of injuries and fractures associated with hip and knees. Hence, in this study, we evaluated morphometric parameters of proximal femur and its association with bone mineral density in pre and post-menopausal women.

The morphometric parameters of proximal femur evaluated in this study were HAL, FAL, FNL, FNW and FNSA. In this study, HAL was found to be  $121.75 \pm 9.63$  mm on the left side and

$118.38 \pm 5.41$  mm on the right side. Similar to this study, Dehghan M *et al* reported HAL of  $118.46 \pm 14.21$  mm while in contrast Bhattacharya *et al* reported HAL of  $260 \pm 4.9$  mm [14, 15].

The value of FAL in the present study was  $105.36 \pm 3.67$  mm on the left and  $97.83 \pm 8.32$  mm on the right side. The result of our study was in accordance to that of Dehghan M *et al* and Bhattacharya *et al* who reported FAL to be  $103.15 \pm 11.18$  mm and  $100.4 \pm 1.03$  mm respectively [14, 15].

The values of FNL and FNW observed on the left side were  $27.44 \pm 3.21$  mm and  $34.81 \pm 3.75$  mm respectively and on the right side the same were  $24.39 \pm 3.15$  mm and  $33.84 \pm 5.24$  mm respectively. The value of FNL observed in this study was slightly lower than that observed in the study of Brazilian cohort [16], while the values of FNW were slightly high.

In this study, FNSA was documented to be  $120.85 \pm 2.95$  degree and  $120.41 \pm 4.06$  degree respectively on right and left side. The result of this study was lower than that observed in Brazilian Cohort which showed the angle to be  $131.81 \pm 5.2$  degree on left side and  $132 \pm 7.2$  degree on right side [16]. However, the finding of our study coincided with that of Kadkhodaei *et al* who showed the angle to be  $121.93 \pm 3.78$  degree [17].

Study of Lavanya Y *et al* [18] showed that 13.3% of premenopausal women and 100% of post-menopausal women were vitamin D deficient. Similar results were also documented by Goswami R *et al* and Harinarayan CV *et al* [19, 20]. In elderly women the reason of low level of vitamin D may be poor diet and decreased exposure to sunlight. As per Mac Laughlin J *et al*, aging reduces the ability of an individual to synthesize vitamin D. There is also decreased hydroxylation of vitamin D and response of intestinal mucosal cells to the serum levels of vitamin D in elderly [21]. Deficiency of vitamin D decreases calcium absorption and accelerates bone loss that increases risk of fracture. Prevalence of vitamin D deficiency in post-menopausal women was reported to be 98% in South Korea, 90% in Japan, 49% in Malaysia and 47% in Thailand [22].

The incidence of hypocalcemia was 23.3% in pre-menopausal women and 60.9% in post-menopausal women in this study which was attributed to low vitamin D levels. Deficiency of vitamin D and calcium may cause secondary hyper-parathyroidism that increases bone turn over and risk of osteoporotic fractures [23]. One of the major causes of low calcium level in post-menopausal women is decreased ovarian function postmenopause that alters calcium metabolism and reduces bone mass. Oestrogen deficiency decreases intestinal absorption and renal reabsorption of calcium thereby inducing calcium loss.

In this study, evaluation of BMD (T-score) showed that there is more incidence of osteoporosis in post-menopausal women (55.4%) compared to premenopausal women (46.6%) which is associated with oestrogen deficiency and age related bone loss. Our findings were in line with that of previous studies which reported that BMD decreases with increase in age. According to Hasager C *et al* [24], there is 3-fold increase in bone loss in post-menopausal period. Similarly, Qureshi HJ *et al* [25] and

Chaudhary S *et al*[26], reported that post-menopausal women are 2 times more prone to have low BMD compared to pre-menopausal women.

In this study, the morphometric parameter of proximal femur was correlated with age, BMI and BMD. Positive non-significant correlation and negative non-significant correlation were observed in case of age and BMI respectively while in case of BMD, negative and significant correlations were documented. Study of Bhattacharya S *et al*[15] showed significant correlation between BMI and pelvic axis length only which was similar to the study of Soltani A *et al*[27]. The effect of BMI on bone morphometry may be direct and indirect. Direct effect is due to increased pressure put on the lower limbs by upper body weight while indirect effect is linked with metabolic disorder resulting in reduced BMD [28]. In contrast, study of Malekzadeh SMetal reported negative correlation of BMI with BMD of femoral neck and FNSA in osteoporotic patients thus highlighting the effect of BMI on proximal femur BMD [29]. GnudiS *et al*[30] documented significant negative correlation between BMD and FNSA which was similar to that observed in this study.

There was also strong positive correlation of BMD with serum calcium and vitamin D in both pre and post-menopausal women. Vitamin D plays important role in calcium and phosphorous homeostasis [31]. It promotes intestinal absorption and renal reabsorption of calcium and phosphorous, thus maintaining their circulatory levels. Low vitamin D also causes compensatory increase in PTH that causes bone resorption to regulate calcium and phosphate. This vitamin D deficiency alters bone structure and density [32].

## Conclusion

Assessment of BMD is a key determinant of bone health in women. Decrease in BMD is not only prevalent in post-menopausal women but also in pre-menopausal women. It suggests that estrogen status and age are not the sole cause for bone loss and osteoporosis. Rather several other associated risk factors such as status of vitamin D and calcium should also be evaluated as they may provide important information on altered metabolism that can lead to low BMD. They can also aid in early diagnosis of osteoporosis and prevention of related fractures.

**Conflict of interest:** Nill

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