THE IMPACT OF DURATION OF MENOPAUSE ON BONE METABOLISM IN DETECTING AND PREVENTING OSTEOPOROTIC FRACTURES

Tirthal Rai
KSHEMA: Nitte University K S Hegde Medical Academy

Rishabh M Hegde (rishabhhegde@gmail.com)
ACI Cumballa Hill Hospital https://orcid.org/0000-0003-0251-2184

Mayur Rai
A J Institute of Medical Sciences and Research Centre

Janice Dsa
A J Institute of Medical Sciences and Research Centre

Srinidhi Rai
Nitte University K S Hegde Medical Academy

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Abstract

ABSTRACT Background: Menopause accelerates bone loss after 10 years of cessation of the menstrual cycle causing osteoporosis. Hip fractures among postmenopausal women escalate morbidity and mortality in these women. Objective: The study was done to evaluate the effect of duration of menopause on BTMs so that it could detect post-menopausal osteoporosis at the earliest and predict the fracture risk. Materials and Methods: The study was conducted in a tertiary hospital in Mangalore on 100 postmenopausal women. The duration of menopause was divided into quartiles. Evaluation and correlation of serum osteocalcin, urinary hydroxyproline, BMI, calcium, phosphorous and alkaline phosphatase was done on the duration of menopause. The subjects comprised 50 osteoporotic and 50 non-osteoporotic post-menopausal women. Continuous variables were represented as median and interquartile ranges. Comparison between two groups was done using the Mann Whitney U test. Comparison between more than two groups was done using the Kruskal Wallis test. The correlation was done using spearman's correlation test. Statistical significance was considered at p<0.05. Results: Serum osteocalcin levels significantly declined and urinary hydroxyproline levels elevated between quartiles of duration of menopause in the entire study group and in osteoporotic women. (p<0.001). There was no significant difference in osteocalcin and hydroxyproline levels between the quartiles in the fracture group. 82% of the osteoporotic had >15 YSM. Conclusion: Osteocalcin levels plateaued after 8 years of menopause and started decreasing after 15 YSM. Osteoporotic fractures were higher in more than 15 YSM and the osteocalcin level was 2.47 ng/ml in this quartile. There is no significant difference in osteocalcin levels in those with fractures, indicating no significance of screening for serum osteocalcin levels once the fractures have occurred. Hence concluding that the duration of menopause is the key indicator for osteoporosis and serum osteocalcin is a potent biomarker for detection of the risk of fracture. Monitoring of serum osteocalcin levels(<2.55ng/ml) after 8 years of menopause is very essential for early prophylactic treatment in order to prevent osteoporotic fractures and the burden associated with it. KEYWORDS: Duration of menopause, osteocalcin, quartiles, urinary hydroxyproline, osteoporotic fractures

Introduction

Menopause is a natural phenomenon that women experience after a mean age of 51 and 48 years in developed and non-developed countries respectively. It is defined as a complete cessation of the menstrual cycle for a period of 12 consecutive months. As age progresses there is a decline in ovarian function due to decreased production of the prime hormone oestradiol and an increase in the follicular stimulating hormone. During this period, women experience varied symptoms, such as hot flushes, sleeplessness, mood swings, vaginal atrophy, and dryness. It also has a marked effect on bone turnover with an accelerated bone loss after 10 years of cessation of the menstrual cycle causing osteoporosis (1). One in every three menopausal women undergo fractures due to osteoporosis and the incidence worldwide is 30–40% whereas in India, osteoporosis occurs 10 years earlier making India the largest affected country with osteoporosis in the world (2).

Osteoporosis is a skeletal deformity characterised by low bone mass, impaired remodelling of the bone causing brittle bones and thus increasing the risk of fractures. Osteoporotic fractures are more commonly seen in the spine, forearm, and hip. Vertebral and distal radius fractures are common in type I or primary osteoporosis whereas hip fractures are common in type II or senile osteoporosis which is seen in women older than 75 years (3). These osteoporotic fractures especially in the hip occur due to trivial falls and increase morbidity and mortality in the elderly. Surgical intervention in the form of either fixation or replacements is usually indicated for these hip fractures. The gold standard for assessment and diagnosing osteoporosis is a DEXA or Bone mineral density scan. Unfortunately, it has many limitations, one of which is that it does not reflect the current bone turnover status at the
time of measurement. Hence it is not a sensitive indicator in monitoring fractures. Instead, bone turnover markers are booming as a potential indicator to assess the risk of osteoporosis in these elderly populations as they are easy to measure and reflect real-time bone metabolic status (4).

Bone turnover comprises of two processes: bone formation by osteoblasts which is measured by osteocalcin (OC), alkaline phosphatase, bone-specific alkaline phosphatase and procollagen products such as C-terminal peptide (P1CP) and N-terminal peptides (P1NP). Bone resorption by osteoclasts is measured with hydroxyproline, pyridinoline [PYD], deoxypyridinoline [DPD], C terminal crosslinking telopeptide of type I collagen, Sclerostin and tartrate-resistant acid phosphatase (5). Off late there has been a lot of debate about the use of Osteocalcin and CTX in being the most promising measure for detecting osteoporotic fractures. Osteocalcin is a non-collagenous protein and has multiple roles with respect to the regulation of calcium homeostasis, metabolic functions, secretion of insulin while also playing a vital role in bone osteology. Osteocalcin levels vary with age, ethnicity, and sex. Levels are noted to be stable in the premenopausal age group and spiked levels in women after menopause. Bone resorption markers like urine hydroxyproline are influenced by dietary changes and are expensive as they are detected by high-performance liquid chromatography and CTX is not bone specific (6). Bone turnover markers can be used to depict the bone microarchitecture and predict the risk of fractures in a cost-effective manner as DEXA is expensive. Women's health is given utmost importance worldwide and with a rise in the aging population, there is a proportional rise in osteoporotic fractures. Thus, the prime aim of our study is to detect the effect of duration of menopause on bone loss so that it could detect primary osteoporosis at the earliest and prevent osteoporotic fractures. Furthermore, this study aims to detect an association of BTMs in predicting the risk of different fracture types, which could not only lead to timely medical intervention but also reduce the need for surgical intervention and the complications associated with it. Hence indirectly tapering economic burden and improving the quality of life in women.

**Materials And Methods**

This cross-sectional study was conducted on 100 menopausal women of which 50 were osteoporotic (post-menopausal women with fractures) and 50 were non-osteoporotic women (post-menopausal women without fractures) visiting the Department of orthopaedics at AJ Institute of Medical Science & Research Centre, Mangalore from June 2010–January 2012. These menopausal women were further divided into two groups based on their duration of menopause; <10 years and ≥10 years of menopause. For further analysis, the duration of menopause was divided into quartiles, Q1 (≤7 years), Q2 (8–14 years), Q3 (15–23 years), and Q4 (>23 years). Approval from the Institution Ethical Committee (AJEC/2009/34) was obtained. The osteoporotic group was subdivided into different types of fractures (hip, spine, and wrist). Osteoporotic fractures were diagnosed primarily with an X-ray. Subjects with diabetes, kidney disease, liver disorder, surgically induced menopause, patients on hormonal therapy, steroidal therapy, and vitamin D supplements, chronic illness, pregnant women, and subjects that sustained a fracture as a result of road traffic accident were excluded. Informed consent was obtained from all the study participants. 5 mL of venous blood sample was collected in a plain vacutainer and centrifuged at 4000 RPM. Serum was then analyzed for OC, alkaline phosphatase (ALP), calcium, and phosphorus. A 24 hours urine sample was collected using HCL as a preservative for hydroxyproline estimation. The sample size for the current study was based on the study done by Kalaiselvi VS and colleagues (7) which comprised 30 osteoporotic women and 30 non-osteoporotic women. The mean serum osteocalcin level in osteoporotic and non-osteoporotic groups was 16.16 ± 4.5 and 11.26 ± 3.07 ng/mL respectively with 80% power and 95% confidence interval. These values were entered into open-source software, OpenEpi, to calculate the sample size. (Dean AG, Sullivan KM, Soe MM. OpenEpi: open...
source epidemiologic statistics for public health; 2011, Available at: www.openepi.com) Thus, the minimum sample size computed was 20 and our sample size was increased to 100

Methods of Estimation

Estimation of serum calcium was done by Arsenazo method (8), inorganic phosphorus by phosphomolybdate method (9), ALP by International Federation of Clinical Chemistry (IFCC)/kinetic method (10), using Agappe reagent kits on Lab Life Robo Chem autoanalyzer and serum OC was estimated by chemiluminescent method (11) in autoanalyzer Siemens’ Immulite 1000 and hydroxyproline by modified Neuman and Logan method (12) in a spectrophotometer using commercially available kits.

STATISTICAL ANALYSIS

Data analysis was done using SPSS version 20. Normality of data was tested using the Kolmogorov Smirnoff test. Categorical variables were represented as frequency and percentage. Continuous variables were represented as mean ± SD for normally distributed data or median and interquartile range for data following skewed distribution. Comparison of continuous variables between two groups was done using the Mann-Whitney U test for skewed distribution. Comparison of continuous variables between more than two groups was done using the Kruskal Wallis test and inter-group comparison was done using the Mann Whitney U test with Bonferroni correction. Correlation between continuous variables was done using Spearman's correlation test. Statistical significance was considered at p < 0.05.

Results

Our study comprised 100 menopausal women from the age group of 50 to 78. Age was higher and BMI was lower in subjects with ≥ 10 years of menopause. (p < 0.05). Serum calcium and osteocalcin levels were significantly lower whereas urinary hydroxyproline was higher in subjects with ≥ 10 years of menopause. (p < 0.05). (Table 1) .88% of the postmenopausal women with fractures (osteoporotic women) had a long duration of menopause of ≥ 10 years and 12% of the osteoporotic women had less than 10 years duration of menopause (Fig. 1).
Table 1
Comparison of various parameters based on duration of menopause

| Parameters                     | Duration of Menopause (in years) | p value |
|-------------------------------|----------------------------------|---------|
|                               | < 10 years (n = 34) | >= 10 years (n = 66) |         |
|                               | Median (Interquartile range)    |         |
| Age (in years)                | 54.00 (49.00–56.25)  | 68.00** (63.00–75.00) | < 0.001 |
| BMI (kg/m²)                   | 22.60 (19.50–23.00)  | 18.30* (17.60–21.93) | 0.001   |
| Age at menarche (in years)    | 13.00 (12.00–13.00)  | 13.00 (12.00–13.00)  | 0.449   |
| Age at menopause (in years)   | 49.00 (46.75–51.00)  | 48.00 (47.00–50.00)  | 0.412   |
| Duration since menopause (in years) | 3.50 (2.00–7.00)  | 20.00** (14.50–27.00) | < 0.001 |
| Serum Calcium (mg/dL)         | 9.20 (8.50–9.70)    | 8.70* (8.20–9.43)    | 0.011   |
| Serum Phosphorus (mg/dL)      | 2.85 (2.50–3.00)    | 2.50 (2.30–3.00)     | 0.091   |
| Serum Alkaline Phosphatase (U/L) | 96.00 (84.00–114.00) | 92.50 (73.00–118.00) | 0.478   |
| Serum Osteocalcin (ng/mL)     | 13.80 (11.90–17.20) | 2.55** (2.00–8.44)   | < 0.001 |
| Urine Hydroxyproline (mg/dL)  | 18.00 (17.00–20.06) | 27.75* (16.87–31.50) | 0.005   |

*, p < 0.05; **, p < 0.001

For further analysis, the duration of menopause was divided into quartiles, Q1 (≤ 7 years), Q2 (8–14 years), Q3 (15–23 years), and Q4 (> 23 years). The majority of the osteoporotic women (82%) had a long duration of menopause of > 15 years. (Fig. 2).

Serum osteocalcin levels significantly declined between quartiles of duration of menopause in the entire study group. (p < 0.001). Among those without fractures, a significant reduction in serum osteocalcin occurred in the second and third quartile compared to the first quartile. (p < 0.001) However, there was no difference in serum
osteocalcin levels between the second and third quartiles. (p = 0.224). There is no significant difference in osteocalcin levels in those with fractures. (Table 2)

Table 2

Comparison of serum osteocalcin in the study subjects

| Serum osteocalcin | Postmenopausal women without fractures | Postmenopausal women with fractures | All subjects |
|------------------|---------------------------------------|------------------------------------|--------------|
|                  | Q1 (12.70–18.11)                      | Q2 (7.36–14.69)                    | Q3 (6.94–13.85) |
| Q1               | 14.44                                 | 9.32<sup>a**</sup>                | 9.32<sup>d**</sup> |
| Q2               | (12.70–18.11)                         | (7.36–14.69)                      | (6.94–13.85)  |
| Q3               | 9.32<sup>a**</sup>                    | 2.19<sup>b**,d**</sup>            | 2.47<sup>c**,e**,f**</sup> |
| Q4               | 9.32<sup>d**</sup>                    | (2.00–2.88)                       | (2.00–2.55)   |
|                  |                                       | (2.00–2.88)                       | (2.00–2.55)   |
|                  |                                       | (2.00–2.47)                       | (2.00–2.55)   |
|                  |                                       | 2.00<sup>c**,e**,f**</sup>        |               |
|                  |                                       | <0.001                             |               |

a, Comparison between Q1 and Q2;
b, comparison between Q2 and Q3;
c, comparison between Q3 and Q4;
d, comparison between Q1 and Q3;
e, comparison between Q1 and Q4;
f, comparison between Q2 and Q4;

*, p < 0.05; **, p < 0.001

In those without fractures, a significant decrease in urinary hydroxyproline was observed in the second and third quartiles in comparison to the first (p < 0.001). However, no significant difference was seen in urinary hydroxyproline between the second and third quartile (p = 0.312). No significant difference was seen in urinary hydroxyproline levels in those with fractures among the quartiles. Among the entire group, there was a significant increase in urine hydroxyproline levels across quartiles. (p < 0.001) (Table 3)
### Table 3
Comparison of urinary hydroxyproline in the study subjects

| Urine hydroxyproline                      | Q1     | Q2     | Q3     | Q4     | p value |
|-------------------------------------------|--------|--------|--------|--------|---------|
| Postmenopausal women without fractures    | 18.00  | 16.50* | 15.50**| —      | 0.001   |
|                                          | (16.62–19.62) | (12.00–17.25) | (11.50–18.50) | —      |         |
| Postmenopausal women with fractures       | 23.75  | 25.00  | 28.50  | 31.50  | 0.144   |
|                                          | (20.00–40.00) | (25.00–35.00) | (24.50–33.50) | (29.50–33.50) |         |
| All subjects                              | 18.00  | 16.50**| 26.00**| 31.50***| < 0.001 |
|                                          | (17.00–20.25) | (12.50–17.50) | (17.75–30.25) | (29.50–33.50) |         |

a, Comparison between Q1 and Q2;
b, comparison between Q2 and Q3;
c, comparison between Q3 and Q4;
d, comparison between Q1 and Q3;
e, comparison between Q1 and Q4;
f, comparison between Q2 and Q4;
*, p < 0.05; **, p < 0.001

Postmenopausal women with wrist, spine, hip and tibial fractures were (n = 17), (n = 12), (n = 20) and (n = 1) respectively. The percentages of spine and hip fractures were higher in the fourth quartile, whereas the overall percentage of hip fracture was higher in patients > 15 years duration period. While the percentage of wrist fractures was higher in the third quartile of menopause. (Fig. 3). There was a significant difference in osteocalcin levels between fracture types in osteoporotic women (p < 0.001). (Table 4)
Table 4
Comparison of biochemical parameters between fracture types in postmenopausal women with fractures.

| Parameters                          | Wrist fracture (n = 17) | Spine fracture (n = 12) | Hip fracture (n = 20) | p value |
|-------------------------------------|-------------------------|-------------------------|-----------------------|---------|
| Median (Interquartile range)        |                         |                         |                       |         |
| Serum Calcium (mg/dL)               | 8.60 a* (8.15–8.70)     | 8.70 b* (8.40–9.70)     | 8.20 (8.00–8.75)      | 0.048   |
| Serum Phosphorus (mg/dL)            | 2.70 (2.40–3.65)        | 2.90 (2.50–3.80)        | 2.50 (2.30–2.88)      | 0.256   |
| Serum Alkaline Phosphatase (U/L)    | 81.00 a*c* (68.00–102.00)| 108.50 (80.00–120.00)  | 110.50 (77.50–127.00)| 0.046   |
| Serum Osteocalcin (ng/mL)           | 2.47 a** (2.09–2.77)    | 2.02 b** (1.92–2.47)    | 2.44 (2.00–2.55)      | 0.001   |
| Urine Hydroxyproline (mg/dL)        | 28.00 (24.50–33.50)     | 31.50 (27.50–40.00)     | 30.25 (28.50–31.50)   | 0.389   |

a, comparison between wrist and spine fracture groups;
b, comparison between spine and hip fracture groups;
c, comparison between wrist and hip fracture groups;
*, p < 0.05; **, p < 0.001

In all subjects, the duration of menopause correlated significantly with age, BMI, calcium, serum osteocalcin, and urinary hydroxyproline. Duration of menopause showed a positive correlation with age (r = 0.964) and urinary hydroxyproline (r = 0.575), while there was a negative correlation with BMI (r=-0.404), calcium (r=-0.244) and serum osteocalcin (r= -0.633). Serum osteocalcin correlated significantly with age, BMI, duration of menopause, serum calcium, and urinary hydroxyproline (p < 0.05). It showed positive correlation with BMI (r = 0.625) and serum calcium level, while a negative correlation was observed with age (r= -0.589), duration of menopause (r= -0.633) and urinary hydroxyproline (r= -0.660). (Table 5).
Table 5
Correlation of duration of menopause, serum osteocalcin, urinary hydroxyproline with various parameters in all subjects

|                         | Age     | BMI      | Duration of menopause | Serum Calcium | Serum phosphorus | Serum ALP | Serum OC | Urinary HP |
|-------------------------|---------|----------|-----------------------|---------------|------------------|-----------|----------|------------|
| **Pearson's coefficient (r)** |         |          |                       |               |                  |           |          |            |
| Duration of menopause   | 0.964** | -0.404** | 1                     | -0.244*       | 0.099            | 0.045     | -0.633** | 0.575**    |
| Serum OC                | -0.589**| 0.625**  | -0.633**              | 0.339**       | -0.136           | -0.100    | 1        | -0.660**   |
| Urinary HP              | 0.521** | -0.592** | 0.575**               | -0.386**      | 0.246*           | 0.294**   | -0.660** | 1          |

Abbreviation: ALP, alkaline phosphatase; HP, hydroxyproline; OC, osteocalcin

*, p < 0.05, **, p < 0.01

The general characteristics and biochemical parameters compared between osteoporotic and non-osteoporotic groups (Table 5)

Higher mean age and longer duration of menopause were observed in osteoporotic women as compared to non-osteoporotic women (p < 0.001). The median BMI in the osteoporotic group was statistically lower as compared to the non-osteoporotic group (p < 0.001). Serum osteocalcin and calcium were significantly higher in non-osteoporotic women as compared to the osteoporotic group whereas the median for urinary hydroxyproline was statistically higher in the osteoporotic women as compared to non-osteoporotic women (p < 0.001). There was no statistical difference in the phosphorus and alkaline phosphatase levels in both groups. (Table 6).
Table 6
Comparison of various parameters between osteoporotic and non osteoporotic groups

| Parameters                                | Postmenopausal women without fractures (n = 50) | Postmenopausal women with fractures (n = 50) | p value |
|-------------------------------------------|-------------------------------------------------|-----------------------------------------------|---------|
| Mean ± SD / Median (Interquartile range)  |                                                 |                                               |         |
| Age (years)                               | 57.92 ± 7.38                                    | 69.10 ± 8.39 **                              | < 0.001 |
| Body Mass Index (kg/m²)                   | 22.75 (19.83–23.95)                             | 18.00 ** (17.53–18.30)                        | < 0.001 |
| Age of Menarche (years)                   | 13.00 (12.00–13.00)                             | 13.00 (12.00–13.00)                           | 0.591   |
| Age of Menopause (years)                  | 49.50 (47.00–51.00)                             | 48.00 (47.00–50.00)                           | 0.061   |
| Duration since Menopause (years)          | 9.00 (3.00–12.00)                               | 22.00 ** (16.00–27.25)                        | < 0.001 |
| Serum Calcium (mg/dL)                     | 9.20 (8.88–9.63)                                | 8.63 ** (8.10–8.83)                           | < 0.001 |
| Serum Phosphorus (mg/dL)                  | 2.80 (2.40–3.00)                                | 2.70 (2.40–3.20)                              | 0.953   |
| Serum Alkaline Phosphatase (U/L)          | 93.00 (81.50–112.50)                            | 101.00 (75.00–118.50)                         | 0.659   |
| Serum Osteocalcin (ng/mL)                 | 12.96 (9.32–15.70)                              | 2.09 ** (2.00–2.55)                           | < 0.001 |
| Urine Hydroxyproline (mg/dL)              | 17.00 (14.38–18.00)                             | 30.13 ** (27.50–33.50)                        | < 0.001 |

*, p < 0.05; **, p < 0.001

Discussion

Estrogen is produced by the ovaries, adrenal glands, and adipose tissue. “Estradiol” is specifically produced by the ovaries while “Estrone” is by the adipose tissues. With menopause there is a decrease in Estradiol. This negates the osteoblastic activity and stimulates osteoclast activity which targets the bone receptors. The prolonged duration of menopause depletes the action of Estradiol and its task is replaced by estrone. Estrogen produced by adipose tissue correlates well with the fat tissues present in a woman (13). Another theory proposes that obesity releases hormones like preptin and amylin from the beta cells of the pancreas along with insulin that stimulates bone formation (14). Thus, implying that an obese woman has a protective role against osteoporosis which was
seconded in our study. Our findings reported low BMI in women with a longer duration of menopause and also in osteoporotic women as compared to non-osteoporotic women. This finding was supported by Morin et al. (15). There are also some studies that refute this finding.

Women undergo two phases of bone loss. In the initial years of menopause, there is a sudden suppression of Estrogen that accelerates the rate of bone resorption coupled with formation, but comparatively the rate of resorption is more than formation. The first phase affects mainly the trabecular bone. However, the second phase, which occurs in late menopause reflects a slower rate of bone formation and accelerated resorption activity. Thus, explaining the decrease in osteocalcin and increase in urinary hydroxyproline in the longer duration of menopause. This is also called age-related bone loss and affects both cortical and trabecular bone. Park et al (16) also found that women with more than 10 years of menopause had significantly lower OC levels than lesser years of menopause. Atalay et al (17) seconded our findings with the highest OC levels in the early menopausal phase. Duration of menopause thus correlates well with BMI, OC, and urinary hydroxyproline. This finding was also supported by Montazerifar et al (18). In our study, the duration of menopause was divided into quartiles for analysing the in-depth effect of the duration of menopause on bone turnover. The majority of the osteoporotic women in our study had >15 years duration of menopause.

Bone is a dynamic tissue that undergoes transformation throughout life with the coordinated action of osteoblasts and osteoclasts. During the embryonic stage, the cartilage is replaced by skeleton bone with the combined effect of these two osteocytes. Infancy to puberty thickness of bone is achieved by osteoblasts and length is achieved by the proliferation of growth plate chondrocytes. Osteoclasts provide shape to the bone during this phase of life and in the third decade, they attain maximum bone mass. After which there is a decline in the rate of bone formation due to Estrogen deficiency leading to negative bone balance thus contributing to osteoporotic fractures (19). Osteocalcin is an osteoblastic marker that is released by the bone matrix and is carboxylated on three glutamine acid residues. It is bone-specific and now a trending marker for detecting osteoporosis (20). In premenopausal women, the median serum OC concentration was 14.4 ng/mL and in postmenopausal women, the median serum OC concentration was 18.6 ng/ml (21). Similar finding was noted in our study with OC level being higher in postmenopausal women without fractures. There are a number of studies describing the same with osteocalcin levels ascending by 50–150% after menopause. However, studies on bone formation at different stages or years of menopause were sparse, OC level in our study slashed significantly between quartiles in all subjects with low OC values in women with more >15 years and highest in less than 7 years of menopause. Among those without fractures (non-osteoporotic women), a significant reduction in serum osteocalcin occurred in the second and third quartile compared to the first. However, there was no difference in serum osteocalcin levels between the second and third quartiles suggesting that the osteoblastic activity plateaus after 8 years of menopause. This may indicate serum osteocalcin to be used as a screening tool for osteoporosis in post-menopausal women. However, in the case of osteoporotic women, there was no significant difference in osteocalcin levels between quartiles, thus low osteocalcin levels demonstrate osteoporosis. Osteocalcin monitoring is irrelevant once the fractures have occurred.

A positive correlation of serum osteocalcin was seen with BMI and calcium in our study whereas a negative correlation was observed with a duration of menopause and age which is in accordance with many studies (22, 23). With the increase in the duration of menopause the serum calcium level decreased. Menopause induces the synthesis of cytokines, monocytes, and T-cells that stimulates osteoclast activity thereby modifying the calcium and phosphate homeostasis. Calcium and phosphorus are the two cardinal macro minerals required for bone building and therefore these correlations are predictable. No significant variation was observed in these parameters among groups with various years since menopause (24). However, in parallel to our findings higher phosphorus,
calcium, and ALP activity have been demonstrated in early menopause (25). Heterogeneity, variabilities in body structure, and varied rate of aging may be responsible for the disparity in these results.

Gurban CV et al (26) propounded those osteoporotic women with longer periods of menopause, presented with higher values of resorption markers. Demirtas O et al (27) emphasized the positive correlation of bone loss and duration of menopause which was supported by the findings in the present study where a significant increase in urinary hydroxyproline across the quartiles, suggesting increased osteoclastic activity causing fragile bones in the elderly population. In those without fractures, a significant decrease in urinary hydroxyproline was observed in the second and third quartiles in comparison to the first as osteoblastic and osteoclastic activity is at equilibrium thus both values dip and plateaus. High urinary hydroxyproline levels were observed in osteoporotic women. Hydroxylation of proline and lysine are required for collagen formation which constitutes bone matrix and hydroxyproline is excreted in the urine during collagen degradation. There are various factors that increase the excretion of hydroxyproline such as vitamin D and calcium deficiency that explains its better correlation with calcium, phosphorus and alkaline phosphatase. Osteocalcin has a negative correlation with hydroxyproline. Therefore, low osteocalcin and high urinary hydroxyproline levels in the later phase of menopause are afflicted with osteoporotic fractures. However, variability in their measurements is obtained by a gelatine rich diet and is non-specific to bony lesions (28). Thus, making osteocalcin a better predictor to detect early osteoporosis.

The maximum strength to the bone is achieved by the combined effect of the inferior cortex that resists compressive loads along with the superior cortex that resists tensile loads. The cortical porosity of the bone is also an important independent factor in providing strength. Failure of this combined effort causes femoral neck fractures. Increased osteoclast activity in menopausal women opens up the Haversian canal forming large macro-pores that are dispersed thus making the cortical layer fragile, incapable to bear weight. The close proximity of these macro-pores reduces the impact on the bone during a trauma (29). The percentages of hip fractures in our study were higher in the third and fourth quartile demonstrating a higher risk of hip fracture in women with > 15 years duration of menopause, followed by wrist and spine fractures. Studies have revealed a two-fold increase in the risk of fractures in the highest quartile (30, 31). Trabecular bone mass reduces by 2.3% and 1.4% in the vertebral column and pelvic bones in the initial years of menopause. However, after 5 years of menopause, the bone mass starts further descending by 10% and 7% in these regions respectively (32). Osteoporotic fractures especially hip fractures are associated with excruciating pain, decreased mobility, and high dependence hence to find a bone turnover marker that could delineate the risk of hip fractures is very essential. There was a significant difference in osteocalcin levels between wrist and spine fractures and spine and hip fractures with the lowest osteocalcin levels observed in the spine fracture group. Urinary hydroxyproline showed no significant difference among the various fracture types. As hip fractures are more prone in subjects with a longer duration of menopause, the osteocalcin level accordingly should recede and urinary hydroxyproline should peak in subjects with hip fracture. However, the disparity in the findings in our study lacked the discovery of a marker in predicting different fractures. This was seconded by many studies where neither bone formation nor bone resorption markers were significantly associated with hip fracture risk in the elderly age group (33, 34). Few studies CTX and NTX to be useful to assess the risk of hip fractures but not osteocalcin (35). The type of fracture sustained cannot be gauged, as it may be due to the grade and impact of fall, along with the applied load to the bones.

**Limitations**

Other resorption markers should have been investigated and correlated to detect the most potent biomarker for primary osteoporosis. Vitamin D levels should have been assessed, as that affects bone formation. Decreased
sample size in osteoporotic fracture group limited the use of BTMS in detecting fracture risk

Conclusion

Duration of menopause showed a positive correlation with urinary hydroxyproline, while there was a negative correlation with BMI, calcium, and serum osteocalcin. Serum osteocalcin levels correlated better with the duration of menopause than urinary hydroxyproline. The decline in the serum osteocalcin levels between quartiles was observed and Osteocalcin levels stagnated at >8 years since menopause and started plunging after 15 years of menopause. The percentages of fractures (spine, hip, and wrist) were higher in >15 years duration of menopause with 95% hip fractures and the osteocalcin level was 2.47 ng/ml in this quartile. There was no significant difference in osteocalcin levels in osteoporotic women (fracture group), indicating It may be irrelevant to screen for serum osteocalcin levels once the fractures have occurred. Thus, indicating that the duration of menopause is a key indicator for osteoporosis, and serum osteocalcin is a potent biomarker for the detection of the risk of fracture. Monitoring of serum osteocalcin levels(<2.55ng/ml) after 8 years of menopause is very essential for early prophylactic treatment in order to prevent osteoporotic fractures and the burden associated with it. However, osteocalcin levels alone do not help in predicting the type of fracture.

Declarations

- Approval from the Institution Ethical Committee (AJEC/2009/34) was obtained
- Consent for publication is Not Applicable
- Availability of data and materials can be made upon a reasonable request from the author
- We declare no Competing interests
- We received no funding from any external source
- TR was part of the team in analysis and evaluation of the samples collected. RH & MR are the treating physicians under whom the patients were admitted, counselled, tested & data was collected. RH is also the corresponding author for the same and helped in writing the manuscript, helped by TR. JD & SR helped in the statistical analysis of the same. All authors have read and approved the final manuscript.

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Figures

Figure 1

Group wise distribution of study subjects based on duration of menopause (in years)
Figure 2

Distribution of subjects based on duration of menopause quartiles
Figure 3

Distribution of fracture types based on duration of menopause quartiles