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ORIGINAL ARTICLE

# Bone mineral density, vertebral fractures and trabecular bone score in primary ovarian insufficiency

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## Abstract

**Purpose** Bone health in primary ovarian insufficiency (POI) is under-investigated. We assessed patients with spontaneous POI for vertebral fractures (VFs) and related parameters of bone health.

**Methods** 70 cases with spontaneous POI (age 32.5 ± 7.0 years) and an equal number of controls were assessed for BMD, TBS, and VFs. BMD at the lumbar-spine (L1-L4), left hip, non-dominant forearm, and TBS (iNsight software) were measured on a dual-energy X-ray absorptiometry (DXA) machine. VFs were assessed by Genant's classification. Serum FSH, LH, estradiol, T4, TSH, iPTH, serum 25(OH)D, total calcium, and inorganic phosphorus were measured.

**Results** BMD at the lumbar-spine, hip and forearm was reduced by 11.5%, 11.4% and 9.1% in POI as compared to controls ($P < 0.001$). Degraded or partially degraded microarchitecture on TBS was observed in 66.7% of patients and 38.2% of controls ($P = 0.001$). 15.7% of the POI patients had VFs, compared to 4.3% of controls ($P = 0.045$). Age, duration of amenorrhea and duration of HRT use were the significant predictors of TBS ($P < 0.01$). Serum 25(OH)D was the significant determinant of VFs. TBS abnormalities were higher in patients with POI and VFs. BMD was not significantly different in patients with and without VFs.

**Conclusion** Thus, lumbar-spine osteoporosis, impaired TBS and VFs were present in 35.7%, 66.7% and 15.7% of patients with spontaneous POI in their early third decade. This indicates need for rigorous investigations for impaired bone health in these young patients and management with HRT, vitamin-D, and possible need for bisphosphonate therapy.

**Keywords** Primary ovarian insufficiency · Absorptiometry · Photon · Bone density · Lumbar vertebrae · Spinal fractures · Hormone replacement therapy · Vitamin D

## Introduction

Primary ovarian insufficiency (POI) is a hypoestrogenic endocrine disorder due to the premature cessation of ovarian function at < 40 years of age. The POI could be syndromic, as in Turner syndrome, due to genetic aberrations or iatrogenic following ovarian surgery, chemotherapy, or radiotherapy [1, 2]. However, most of the cases are idiopathic and have spontaneous POI with an increased prevalence of coexistent thyroid autoimmunity [3]. The usual age at presentation of patients with idiopathic POI is less than 30 years [4]. The prolonged estrogen deficiency can have adverse effects on bone health, as indicated by low bone mineral density (BMD) and osteoporosis in them [5–12].

Vertebral, hip and forearm fractures are the major causes of morbidity and mortality in osteoporosis. Multiple factors influence the risk of fractures in osteoporosis, including age, tendency to fall, coexisting illnesses, medications, and premature estrogen loss, as in POI. Recently, poor bone microarchitecture has been identified as an independent risk factor for fragility fractures, in addition to low BMD. Trabecular bone score (TBS) is a newly developed non-invasive tool to assess the trabecular microarchitecture of the lumbar spine [12, 13]. Several disorders like

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type 2 diabetes mellitus, congenital adrenal hyperplasia on steroids, acromegaly, Cushing disease and hypoparathyroidism show impaired TBS with bone fragility [15–18].

The functional impact of osteoporosis as indicated by vertebral fractures (VF) and poor TBS has not been investigated earlier in young patients with spontaneous POI. Here, we report on the prevalence of osteoporosis, VFs, impaired TBS and their determining factors in patients with POI.

## Materials and methods

Patients were 70 women with idiopathic POI attending the Reproductive and Endocrine clinics of Maulana Azad Medical College (New Delhi, India) from 2018 to 2021. The diagnosis of POI was based on amenorrhea for at least four months duration, age < 40 years, and serum follicle-stimulating hormone (FSH) levels of > 25 IU/L documented on two occasions at least four weeks apart [19]. Patients with age < 18 years, Turner's syndrome and those with POI due to iatrogenic causes were excluded. Patients on long-term steroids or antiepileptic drugs and those with TSH > 10 mIU/L were also excluded from the study. The number of years of normal menstruation was noted for all of them. The duration of POI was calculated by subtracting the age at the onset of amenorrhea from the current age. In cases with primary amenorrhea, this duration was determined by subtracting 13.5 years from the current age of the patients [20]. All the patients were assessed for a history of smoking, and their past history of fragility fractures at the DXA assessment. Their physical activity status was assessed by the International Physical Activity Questionnaire (IPAQ) [21]. Daily dietary calorie, calcium and phytate intake was calculated by a trained dietician using an open-ended, semi-quantitative food frequency questionnaire incorporating information on seven food groups and 40 common Indian food items [22, 23].

Twenty-seven of the 70 cases were newly diagnosed with no prior history of hormone replacement therapy (HRT). Forty-three patients were already under follow-up at our clinic and were on HRT. The HRT regimen prescribed for them was 0.625 mg-1.25 mg of oral conjugated equine estrogen (day 1 to 25) and 10 mg of medroxyprogesterone acetate (day 15 to 25) each month. These patients were also prescribed oral calcium-carbonate supplementation twice a day with meals, along with 60,000 IU of oral cholecalciferol once a month. Each tablet of calcium carbonate contained 500 mg of elemental calcium. Thirty-two of the 43 cases who were on HRT had received it for > 1 year with a median duration of 21 months. Eleven of them had received HRT for < 1 year.

### Controls

The control group included age-matched (± 2 years) healthy women > 18 years of age who had normal menstrual cycles and consented to undergo dual-energy X-ray absorptiometry (DXA). They were recruited from the hospital's nursing staff in a 1:1 ratio with patients. Part of the control group was selected among the patients' relatives since the entire control group could not be drawn among the hospital staff. Controls with a spot blood glucose level of > 7.8 mmol/L, and TSH > 10 mIU/L were excluded.

All the patients and controls were called on a prescheduled date for assessment of biochemical and hormonal parameters, BMD, TBS and vertebral fractures.

### Bone mineral density, trabecular bone score and assessment of vertebral fractures

BMD was measured at the lumbar spine (L1-L4), left hip, and non-dominant forearm using dual-energy X-ray absorptiometry (DXA) (Discovery A 84,023; Hologic Inc., MA) as per the International Society for Clinical Densitometry's (ISCD) criteria [14]. The coefficients of variation for the lumbar spine, hip, femur neck, and distal forearm, respectively, were 0.69%, 1.73%, 1.40%, and 1.39%. Lumbar vertebrae with fractures were not included for BMD analysis of the lumbar spine.

TBS was measured using the iNsight software (version 3.0.2.0, Med-Imaps, France) installed on the DXA machine. The software assesses the gray-level texture using pixels collected during BMD assessment of L1-L4 spine with a scan time of 30 s per subject. TBS represents the average L1-L4 TBS and is a unitless measurement [24]. As per ISCD guidelines, TBS was not assessed for patients < 20 years of age or with a BMI > 37 kg/m$^2$ [14]. Lumbar vertebrae, if excluded from the BMD analysis, were also excluded from the TBS assessment. The TBS > 1.310 reflects denser trabeculae with good microarchitecture connectivity, while the TBS < 1.230 indicates degraded microarchitecture with high susceptibility to VF. A score between 1.310 and 1.230 indicates partially degraded microarchitecture [24, 25].

A lateral view of the spine was obtained by rotating the arm of the DXA machine. The VFA software calculated the severity of VF based on six points marked on the anterior, posterior, and middle of the superior and inferior surfaces of each vertebra. Developmental variations, degenerative changes, and syndesmophytes were excluded during the marking of these points. The ratios of anterior and posterior vertebral heights were used to compute wedge and biconcave deformities. The ratios of the anterior, middle,

and posterior heights of the adjacent vertebrae were used to compute the crush deformity. Vertebral fractures were graded according to Genant's classification [26]. Grade 1 VFs indicated a 20% to 24.9% reduction in vertebral height, grade 2 VFs indicated a 25% to 39.9% reduction, and grade 3 VFs indicated a 40% or more reduction. The assessment of VFs was limited to T7 to L4 level because of poor visualization of T4 to T6 vertebrae in several patients due to overlapping scapula and ribs [27, 28].

### Biochemical parameters

Serum FSH, LH, estradiol, T4, TSH, and iPTH were measured by chemiluminescence (Elecsys-2010, Roche, Germany). Serum 25(OH)D was measured by chemiluminescence (LAISON, DiaSorin, Inc., MN) with the coefficient of variation of 2.9–5.5%. Serum total calcium and inorganic phosphorus were measured on the Hitachi 917, Roche, Germany (normal ranges: 2.02–2.59 mmol/L and 0.81–1.45 mmol/L, respectively). The intra-assay and inter-assay coefficients of variation were 3.5–5.0%.

### Statistical analysis

The data is presented as mean (SD) and median, with interquartile range (IQR). Student's t test was used to compare continuous parameters with normal distribution. Comparison for factors that were not normally distributed was performed using the Mann–Whitney U test. The difference in the frequency of various parameters was analysed by Fisher's exact test. Age, BMI, duration of amenorrhea, duration of HRT, serum 25(OH)D, iPTH, estrogen, and serum FSH levels were used as independent factors in multiple regression analysis to determine the factors related to TBS, VFs, and low BMD in POI. Statistical analysis was performed using the SPSS-20.0 program. P value $< 0.05$ was considered significant.

The institutional ethics committee approved the study protocol and written informed consent was obtained from each study subject for DXA and biochemical investigations. (F.No. 17/IEC/MAMC/2017/OBG/06).

## Results

Table 1 shows the clinical, biochemical, hormonal and DXA characteristics of POI patients and the control group. The two groups were comparable for age and BMI. The mean values of serum FSH and LH were high and estradiol was low in the POI group [83.3 ± 37.7 IU/L, 43.2 ± 17.4 IU/L and 67.16 (18.35–111.2) pmol/L, respectively]. Fifteen (21.4%) patients in the POI group had primary amenorrhea, and 55 (78.6%) had secondary amenorrhea. The median duration of amenorrhea was 5.0 (2.0–8.8) years. Five patients had primary hypothyroidism but were euthyroid on thyroxine replacement. None of the POI patients had adrenal insufficiency or features of metabolic bone disease, with serum cortisol being normal in all of them. Only two patients in the POI group and none in the control group were smokers. There was a history of bone fracture in four of the seventy POI patients (5.7%) and two controls (2.9%). However, all the fractures were preceded by significant trauma, and none had a fragility fracture. The mean serum total calcium was higher in the POI group as compared to controls. The mean serum inorganic phosphate and iPTH and the median value of serum 25(OH)D of POI patients were within the normal range and comparable to those of the controls (Table 1).

### BMD, vertebral fractures and TBS in POI

The mean BMD values at the lumbar spine, hip, and forearm were lower in the POI group than in controls, with average reductions being 11.5%, 11.4% and 9.1% respectively ($P < 0.001$ for all). Z-score analysis revealed a significantly higher proportion of POI patients having osteoporosis than the controls at all sites (Table 1). The difference was most marked at the forearm, where 15.2% of POI patients had osteoporosis in contrast to none of the controls ($P < 0.01$).

Table 2 shows the differences in clinical characteristics of POI patients with and without osteoporosis. BMI, years of menstruation prior to amenorrhea and duration of HRT used were significantly lower in POI patients with osteoporosis. The age, duration of amenorrhea, serum FSH and estradiol were comparable in POI with and without osteoporosis. Similar results were obtained on analysing for osteoporosis at the hip and forearm. The mean daily dietary calcium intake of 70 patients with POI was low (432 ± 194 mg), and their mean MET score was in the moderate category (1241 ± 363). Both these parameters were comparable between POI patients with and without lumbar spine Z- score $< -2.0$ and $\geq -2.0$, with and without VFs, and those with degraded and non-degraded TBS (Tables 2, 3, 4).

Prevalence of VFs [11/70 (n = 15.7%) vs. 3/70 (4.3%), $P = 0.045$, Table 1] and the proportion of subjects with multiple or grade 2 VFs tended to be higher in POI than in the control group ($P = 0.05$ and 0.07 respectively, Table 1). POI patients with VFs had significantly lower mean serum 25(OH)D than those without VFs (28.5 ± 19.2 nmol/L vs 61.9 ± 44.7 nmol/L, $P < 0.001$, Table 3). The mean age and BMD did not differ significantly between POI patients with and without VFs.

**Table 1** Comparison of clinical, biochemical characteristics, bone mineral density, trabecular bone score, and vertebral fractures in POI patients and controls

| Characteristic | POI patients (n = 70) | Controls (n = 70) | P |
|---|---|---|---|
| Age (years) | 32.5 ± 7.0 | 32.7 ± 6.9 | 0.91 |
| BMI (kg/m$^2$) | 26.3 ± 5.4 | 26.0 ± 4.5 | 0.66 |
| Serum total calcium (mmol/L) | 2.34 ± 0.1 | 2.29 ± 0.14 | 0.01 |
| Serum inorganic phosphate (mmol/L) | 1.21 ± 0.19 | 1.22 ± 0.20 | 0.74 |
| Serum iPTH (ng/L) | 63.8 ± 29.4 | 60.4 ± 29.4 | 0.50 |
| Serum 25(OH)D (nmol/L) | 46.3 (22.28–76.5) | 32.95(17.53–65.77) | 0.09 |
| BMD (g/cm$^2$) | | | |
| L1-4 AP spine | 0.85 ± 0.12 | 0.96 ± 0.12 | < 0.001 |
| Femoral neck | 0.65 ± 0.10 | 0.75 ± 0.12 | < 0.001 |
| Trochanter | 0.59 ± 0.08 | 0.66 ± 0.08 | < 0.001 |
| Total hip | 0.78 ± 0.13 | 0.88 ± 0.11 | < 0.001 |
| Ultradistal-forearm | 0.37 ± 0.06 | 0.43 ± 0.05 | < 0.001 |
| Mid-forearm | 0.54 ± 0.06 | 0.58 ± 0.04 | < 0.001 |
| Proximal-forearm | 0.61 ± 0.06 | 0.66 ± 0.06 | < 0.001 |
| Total forearm | 0.50 ± 0.05 | 0.55 ± 0.04 | < 0.001 |
| Osteoporosis (Z score < – 2.0) | | | |
| Lumbar spine (n, %) | 25 (35.7%) | 8 (11.4%) | < 0.01 |
| Hip region (n, %) | 14 (20%) | 3 (4.3%) | 0.01 |
| Total forearm (n, %)[a] | 10 (15.2%) | 0 (0%) | < 0.01 |
| Trabecular bone score[b] | | | |
| Mean ± SD | 1.28 ± 0.08 | 1.34 ± 0.08 | < 0.001 |
| < 1.230 (n, %) | 18 (27.3%) | 7 (10.3%) | |
| 1.230–1.310 (n, %) | 26 (39.4%) | 19 (27.9%) | |
| > 1.310 (n, %) | 22 (33.3%) | 42 (61.8%) | 0.002 |
| Vertebral fractures | | | |
| Number of subjects with fractures (n, %) | 11 (15.7%) | 3 (4.3%) | 0.045 |
| Single fractures | 9 (12.9%) | 2 (2.8%) | |
| Multiple fractures | 2 (2.9%) | 1 (1.4%) | 0.05 |
| Grade 1 fractures | 5 (7.1%) | 2 (2.9%) | |
| Grade 2 fracture[c] | 6 (8.6%) | 1 (1.4%) | 0.07 |

[a]Patients with age less than 18 years were not included for analysis of Z-score at forearm

[b]Patients analyzed for TBS (n = 66)

[c]Patients with multiple fractures of different grades were shown against the higher grade

TBS was analysed in 66 of 70 POI cases who were > 20 years of age. The mean TBS was significantly lower and the percentage of patients with degraded microarchitecture was significantly higher in POI group than controls (P < 0.01, for both, Table 1). The mean age of POI patients with degraded TBS was higher than that of POI patients with normal TBS (35.5 ± 5.6 years vs. 29.2 ± 5.1 years, P < 0.001, Table 4). Interestingly, degraded TBS was present in a higher proportion of POI patients with VFs than those without VFs (P = 0.01).

Table 5 shows a comparison of various parameters between 43 patients with and 27 without HRT use. The frequency of subjects with BMD Z-score < − 2.0 at lumbar spine was significantly higher in those without any HRT use as compared to those with a history of HRT use (51.9% vs. 25.6%, P = 0.04). The mean BMD at the ultra-distal forearm was higher in the POI group using HRT. However, there was no difference in the frequency of VFs and TBS in the stratified analysis between patients with and without HRT use.

The mean serum total calcium levels were comparable between patients with lumbar spine Z- score < − 2.0 and ≥ − 2.0 (Table 2), with and without VFs (Table 3) and those with degraded and non-degraded TBS (Table 4). Serum phosphate was higher in POI patients with lumbar spine Z- score < − 2.0 and ≥ − 2.0 (1.29 ± 0.15 mmol/L vs 1.17 ± 0.20 mmol/L, P = 0.02), but comparable in patients with and without VFs (Table 3) and with degraded and non-degraded TBS (Table 4).

**Table 2** Comparison of clinical, biochemical parameters and TBS in POI patients with lumbar spine Z-score < −2.0 or ≥ −2

| Characteristic | Lumbar spine BMD—Z-score | | P |
|---|---|---|---|
| | < −2.0 (n = 25) | ≥ −2.0 (n = 45) | |
| Age (year) | 30.5 ± 7.3 | 33.7 ± 6.6 | 0.07 |
| BMI, kg/m$^2$ | 23.6 ± 4.6 | 27.8 ± 5.2 | < 0.01 |
| Years of normal menstruation | 9.7 ± 8.8 | 14.7 ± 7.9 | 0.02 |
| Duration of amenorrhea (year) | 7.4 ± 5.2 | 5.5 ± 4.3 | 0.09 |
| FSH (IU/L) | 79.8 ± 37.5 | 85.3 ± 38.0 | 0.56 |
| Estradiol (pmol/L) Median (IQR) | 51.4 (18.3–113.4) | 67.9 (22.7–112.3) | 0.37 |
| Serum iPTH (ng/L) | 67.8 ± 25.3 | 61.6 ± 31.6 | 0.40 |
| Serum 25(OH)D (nmol/L) | 48.5 ± 27.0 | 61.5 ± 50.0 | 0.17 |
| Serum total calcium (mmol/L) | 2.35 ± 0.08 | 2.33 ± 0.11 | 0.61 |
| Serum inorganic phosphate (mmol/L) | 1.29 ± 0.15 | 1.17 ± 0.20 | 0.02 |
| Mean dietary calcium (mg/day) | 419 ± 157 | 439 ± 213 | 0.68 |
| Total MET Score | 1279 ± 370 | 1221 ± 361 | 0.53 |
| Duration of HRT use (months) Median (IQR) | 0 (0–12) | 12 (0–24) | 0.02 |
| Z-score < −2 for total hip (n, %) | 9 (36%) | 5 (11.1%) | 0.01 |
| Z-score < −2 for total forearm[a] (n, %) | 7 (31.8%) | 3 (6.8%) | 0.03 |
| Trabecular bone score[b] | | | |
| Mean ± SD | 1.24 ± 0.07 | 1.30 ± 0.08 | < 0.01 |
| < 1.230 (n, %) | 11 (50%) | 7 (15.9%) | |
| 1.230–1.310 (n, %) | 7 (31.8%) | 19 (43.2%) | |
| > 1.310 (n, %) | 4 (18.2%) | 18 (40.9%) | 0.01 |

[a]Patients with age less than 18 years were not included for analysis of Z-score at forearm

[b]Patients with age less than 20 years were not included for analysis of TBS

### Multivariate analysis for factors determining BMD, VFs and TBS in POI

The total duration of amenorrhea, use of HRT, and BMI were the significant determinants of BMD (P < 0.001 for all, Table 6). BMD decreased by 0.013 g/cm$^2$ for each year of increased duration of amenorrhea. Lumbar BMD increased by 0.002 g/cm$^2$ with each month increase of HRT (P < 0.001). Lumbar BMD also increased by 0.009 g/cm$^2$ per 1 kg/m$^2$ increase in BMI.

Serum 25(OH)D was the only significant determinant of VFs (P = 0.03). POI patients with serum 25(OH)D < 25 nmol/L had a 42% higher risk of VFs than those with levels above 25 nmol/L (P = 0.03). The odds of VFs were reduced by 9% for every 2.5 nmol/L increase in serum 25(OH)D (odds ratio = 0.910, 95% CI 0.837–0.988).

The current age, duration of amenorrhea, and HRT use were the significant predictors of TBS in POI patients (Table 6). TBS in patients with POI decreased by 0.005 and 0.009 for every year of increase in age and duration of amenorrhea, respectively (P < 0.001 for both). TBS improved by 0.001 with each month of HRT use (P = 0.009).

## Discussion

This study provides novel information on prevalence of osteoporosis and more importantly, its functional relevance in terms of VFs in young patients with POI. Other novel information in this study is the bone microarchitecture assessment by TBS, its interrelationship with VFs and their determinants in POI.

This study shows that patients with POI had reduced BMD compared to their normal peers. One-third of them had a Z-score indicating osteoporosis at the lumbar-spine in the early third decade of life. The duration of amenorrhea was the significant determinant of low BMD with a longer duration of HRT being protective. These observations are akin to those reported earlier by other investigators [6, 11]. Bagur et al. observed a higher loss of BMD at the lumbar-spine with increasing duration of amenorrhea, especially in non-obese patients with POI [11]. Similarly, Leite-Silva et al. observed low BMD in the lumbar and hip regions in POI patients at an average age of 29 years [6].

The present study indicated that 16% of patients with POI already had VFs at a young age, after an average of five years

**Table 3** Clinical, biochemical parameters, BMD and TBS in POI patients with and without VFs

| Characteristic | Vertebral fractures | | P |
|---|---|---|---|
| | Present (n = 11) | Absent (n = 59) | |
| Age (year) | 34.1 ± 5.0 | 32.3 ± 7.2 | 0.31 |
| BMI (kg/m$^2$) | 28.2 ± 1.0 | 26.0 ± 5.4 | 0.20 |
| Years of normal menstruation | 13.7 ± 8.6 | 12.7 ± 8.6 | 0.72 |
| Duration of amenorrhea (year) | 6.4 ± 5.4 | 6.1 ± 4.6 | 0.88 |
| FSH (IU/L) | 71.2 ± 33.5 | 84.3 ± 39.8 | 0.31 |
| Estradiol (pmol/L) Median (IQR) | 73.4 (18.3–114.1) | 58.7 (18.3–110.1) | 0.75 |
| Serum iPTH (ng/L) | 76.8 ± 36.8 | 61.4 ± 27.6 | 0.11 |
| Serum 25(OH)D (nmol/L) | 28.5 ± 19.2 | 61.9 ± 44.7 | < 0.001 |
| Serum total calcium (mmol/L) | 2.32 ± 0.06 | 2.34 ± 0.11 | 0.44 |
| Serum inorganic phosphate (mmol/L) | 1.20 ± 0.18 | 1.21 ± 0.19 | 0.95 |
| Mean dietary calcium (mg/day) | 416 ± 140 | 434 ± 203 | 0.77 |
| Total MET Score | 1311 ± 285 | 1228 ± 276 | 0.49 |
| Duration of HRT use (months) Median (IQR) | 8 (0–18) | 12 (0–24) | 0.62 |
| Mean BMD, g/cm$^2$ | | | |
| L1-4 AP spine | 0.83 ± 0.10 | 0.85 ± 0.13 | 0.51 |
| Total hip | 0.79 ± 0.10 | 0.78 ± 0.14 | 0.75 |
| Femoral neck | 0.64 ± 0.08 | 0.65 ± 0.10 | 0.58 |
| Trochanter | 0.59 ± 0.06 | 0.59 ± 0.08 | 0.80 |
| Total forearm | 0.50 ± 0.04 | 0.51 ± 0.06 | 0.88 |
| Ultra-distal forearm | 0.37 ± 0.05 | 0.37 ± 0.07 | 0.86 |
| Mid-forearm | 0.54 ± 0.04 | 0.54 ± 0.06 | 0.94 |
| Proximal forearm | 0.60 ± 0.04 | 0.61 ± 0.06 | 0.51 |
| Trabecular bone score | | | |
| Mean ± SD | 1.25 ± 0.03 | 1.28 ± 0.09 | 0.06 |
| < 1.230 (n, %) | 2 (18.2%) | 16 (29.1%) | |
| 1.230–1.310 (n, %) | 9(81.8%) | 17 (30.9%) | |
| > 1.310 (n, %) | 0 (0%) | 22 (40%) | 0.003 |

of amenorrhea. These fractures occurred despite a higher mean serum calcium level in the POI group. Interestingly, all the VFs were newly detected, and none of these patients had backaches or other suggestive symptoms. Despite their asymptomatic nature, the high prevalence of VFs in POI at a relatively younger age is likely to have clinical significance. The presence of VFs promotes further fractures, lumbar-spine instability, and is the leading cause of morbidity in both men and women with osteoporosis [29]. The benefit of adding anti-osteoporosis treatment such as bisphosphonates along with HRT in POI with osteoporosis to prevent fractures and their effect on existing fractures needs to be assessed on priority in a research setting.

In this study, though BMD increased with the use of HRT, the mean duration of HRT use was comparable between patients with and without VFs. This indicated that a subset of patients with POI either already had VFs before using HRT or developed these fractures despite using HRT. To the best of our knowledge, there has been no previous study on the determinants of VFs in young-onset POI. Among various factors assessed, serum 25(OH)D was the significant determinant of VFs. The mean serum 25(OH)D of POI patients with VFs (28.1 nmol/L) was in the vitamin D deficient range (< 30.0 nmol/L). In contrast, the serum 25(OH)D of POI patients without VFs (61.9 nmol/L) indicated vitamin D sufficiency, i.e., > 50 nmol/L [30]. Though, mean serum calcium and 25(OH) D in POI patients with lumbar spine Z- score $< -2.0$ and $\geq -2.0$ were comparable, serum phosphate levels were higher in patients with Z- score $< -2.0$. This difference in the mean serum phosphate could be incidental and needs to be confirmed in further studies. Interestingly, BMD, including Z-score analysis, could not differentiate patients with and without VFs.

**Table 4** Clinical, biochemical parameters and BMD in POI patients with TBS < 1.31 and TBS ≥ 1.31

| Characteristic | Trabecular bone score in POI[a] | | P |
|---|---|---|---|
| | < 1.31 (n = 44) | ≥ 1.31 (n = 22) | |
| Age (year) | 35.5 ± 5.6 | 29.2 ± 5.1 | < 0.001 |
| BMI (kg/m$^2$) | 27.2 ± 5.0 | 26.0 ± 5.3 | 0.41 |
| Years of normal menstruation | 15.0 ± 8.1 | 11.0 ± 7.6 | 0.06 |
| Duration of amenorrhea (year) | 7.0 ± 4.9 | 4.8 ± 4.3 | 0.08 |
| FSH (IU/L) | 76.5 ± 31.2 | 96.8 ± 48.0 | 0.08 |
| Estradiol (pmol/L) Median (IQR) | 72.7 (22–117.4) | 56.1 (18.3–97.6) | 0.26 |
| Serum iPTH (ng/L) | 63.7 ± 32.1 | 61.6 ± 24.3 | 0.79 |
| Serum 25(OH)D (nmol/L) | 50.9 ± 37.2 | 71.5 ± 54.5 | 0.08 |
| Serum total calcium (mmol/L) | 2.36 ± 0.08 | 2.32 ± 0.11 | 0.18 |
| Serum inorganic phosphate (mmol/L) | 1.20 ± 0.15 | 1.20 ± 0.17 | 0.95 |
| Mean dietary calcium (mg/day) | 410 ± 136 | 479 ± 268 | 0.17 |
| Total MET Score | 1225 ± 367 | 1238 ± 364 | 0.90 |
| Duration of HRT use (months) Median (IQR) | 12 (0–22.5) | 12 (0–24) | 0.87 |
| Mean BMD, g/cm$^2$ | | | |
| L1-4 AP spine | 0.82 ± 0.11 | 0.93 ± 0.11 | < 0.001 |
| Total hip | 0.76 ± 0.14 | 0.84 ± 0.10 | 0.02 |
| Femoral neck | 0.63 ± 0.09 | 0.71 ± 0.09 | < 0.01 |
| Trochanter | 0.57 ± 0.08 | 0.64 ± 0.07 | < 0.01 |
| Total forearm | 0.50 ± 0.05 | 0.53 ± 0.05 | 0.01 |
| Ultra-distal forearm | 0.36 ± 0.06 | 0.41 ± 0.06 | 0.004 |
| Mid-forearm | 0.53 ± 0.06 | 0.56 ± 0.05 | 0.05 |
| Proximal forearm | 0.60 ± 0.06 | 0.64 ± 0.05 | 0.02 |

[a]Patients with age less than 20 years were not included for analysis of TBS

The present study revealed that bone microarchitecture, as assessed by TBS, was impaired in two thirds of patients with POI. Duration of amenorrhea and higher age were the significant determinants of their degraded TBS, with HRT being protective. There is only one earlier study reporting TBS in 60 young patients with POI [31]. Low TBS was observed in 44% of the patients with spontaneous POI and in 17% of the patients with POI due to iatrogenic causes. The higher prevalence of abnormal TBS in two thirds of POI patients could be explained by the fact that most of them had spontaneous POI occurring against the background of a high prevalence of vitamin D deficiency. In our earlier studies, vitamin D deficiency has been found to be common among indoor residents of Delhi [32, 33].

To the best of our knowledge, there has been no previous study assessing the interrelationship of TBS with VFs in POI patients. Interestingly, the present study revealed degraded TBS in all the POI patients with VFs, with none having normal values. In contrast, BMD was not significantly different in POI patients with and without VFs. The occurrence of VFs in POI without significant alteration in BMD, but with significantly impaired TBS, is similar to that reported in several other endocrine disorders, i.e., type 2 diabetes, glucocorticoid induced osteoporosis, acromegaly, and hypoparathyroidism [13–16]. However, as in these disorders, abnormalities in TBS alone would not explain VFs in POI because 60% of POI patients without VFs also had subnormal TBS.

To conclude, the present study has several practical implications for assessing bone health in patients with POI. The presence of lumbar spine osteoporosis, impaired TBS, and VFs in 35.7%, 66.7% and 15.7% of patients, respectively, indicates the need for TBS and vertebral fracture assessment along with BMD measurement to assess bone health in POI. Furthermore, the association between VFs and serum 25(OH)D indicates that all POI cases require regular supplementation to maintain normal vitamin D status. The effect of bisphosphonates after correction of hypovitaminosis D on BMD and TBS in POI could be a subject of further studies.

**Table 5** BMD, TBS, Z-score and vertebral fractures in POI patients with and without HRT

| Parameter | History of Prior HRT | | P value |
|---|---|---|---|
| | Yes (n = 43) | No (n = 27) | |
| Age (year) | 32.3 ± 7.2 | 32.9 ± 6.6 | 0.74 |
| BMI (kg/m$^2$) | 25.7 ± 5.5 | 27.3 ± 5.1 | 0.22 |
| Serum total calcium (mmol/L) | 2.34 ± 0.11 | 2.33 ± 0.08 | 0.73 |
| Serum inorganic phosphate (mmol/L) | 1.23 ± 0.18 | 1.17 ± 0.20 | 0.14 |
| Serum iPTH (ng/L) | 59.48 ± 31.23 | 70.67 ± 25.40 | 0.12 |
| Serum 25(OH)D (nmol/L) | 24.29 ± 17.24 | 20.21 ± 17.70 | 0.34 |
| FSH (IU/L) | 81.06 ± 34.60 | 86.99 ± 42.46 | 0.53 |
| Osteoporosis (Z score < −2.0) | | | |
| Lumbar spine (n, %) | 11 (25.6%) | 14 (51.9%) | 0.04 |
| Hip region (n, %) | 8 (18.6%) | 6 22.2%) | 0.76 |
| Total forearm (n, %) | 4 (9.8%) | 6 (24%) | 0.17 |
| Mean BMD, g/cm$^2$ | | | |
| L1-4 AP Spine | 0.86 ± 0.11 | 0.83 ± 0.13 | 0.25 |
| Total hip | 0.80 ± 0.11 | 0.75 ± 0.16 | 0.16 |
| Femoral neck | 0.66 ± 0.10 | 0.64 ± 0.09 | 0.29 |
| Trochanter | 0.60 ± 0.09 | 0.59 ± 0.07 | 0.64 |
| Total forearm | 0.52 ± 0.05 | 0.49 ± 0.06 | 0.06 |
| Ultradistal forearm | 0.39 ± 0.06 | 0.35 ± 0.05 | 0.03 |
| Mid Forearm | 0.55 ± 0.06 | 0.52 ± 0.06 | 0.07 |
| Proximal forearm | 0.61 ± 0.05 | 0.61 ± 0.06 | 0.65 |
| Trabecular bone score | | | |
| Mean ± SD | 1.28 ± 0.08 | 1.27 ± 0.07 | 0.68 |
| < 1.230 (n, %) | 11 (26.8%) | 7 (28%) | |
| 1.230–1.310 (n, %) | 17 (41.5%) | 9 (36%) | |
| > 1.310 (n, %) | 13 (31.7%) | 9 (36%) | 0.90 |
| Vertebral fractures | | | |
| No of subjects with fractures (n, %) | 7 (16.3%) | 4 (14.8%) | 0.99 |
| Single fracture (n, %) | 7 (16.3%) | 2 (7.4%) | |
| Multiple fractures (n, %) | 0 | 2 (7.4%) | 0.11 |
| Grade 1 fractures (n, %) | 4 (9.3%) | 1 (3.7%) | |
| Grade 2 fractures (n, %) | 3 (7%) | 3 (11.1%) | 0.55 |

**Table 6** Regression model for bone mineral density, trabecular bone score and vertebral fractures in POI patients

| Regression model for BMD in POI patients | | | | |
|---|---|---|---|---|
| Characteristics | B coefficient | P value | 95% confidence interval | |
| BMI (kg/m$^2$) | 0.009 | < 0.001 | 0.004 | 0.013 |
| Duration of amenorrhea | −0.013 | < 0.001 | −0.183 | −0.007 |
| Months of HRT used | 0.002 | < 0.001 | 0.001 | 0.003 |
| Constant | 0.663 | < 0.001 | 0.537 | 0.789 |
| Regression model for TBS in POI patients | | | | |
| Characteristics | B coefficient | P value | 95% confidence interval | |
| Age | −0.005 | < 0.001 | −0.008 | −0.002 |
| Duration of amenorrhea | −0.009 | < 0.001 | −0.013 | −0.005 |
| Months of HRT used | 0.001 | 0.009 | 0.0002 | 0.002 |
| Constant | 1.487 | < 0.001 | 1.388 | 1.587 |
| Logistic regression model for VFs in POI patients | | | | |
| Characteristics | Odds Ratio | P value | 95% confidence interval | |
| Vitamin D levels (nmol/L) | 0.910 | 0.025 | 0.837 | 0.988 |
| Constant | 0.871 | 0.827 | 0.254 | 2.988 |

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**Data availability** All datasets generated during and/or analysed during the current study are not publicly available but will be available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** The authors have no competing interests to declare that are relevant to the content of this article.

**Ethical approval** The study was performed in accordance with the ethical standards as laid down in the declaration of Helsinki, and was approved by the Institutional Ethics Committee of Maulana Azad Medical College, New Delhi, India.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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