Abstract

Introduction: Neurofibromatosis type 1 (NF1) is an autosomal dominant neurocutaneous disease characterised by multisystemic involvement, including bone tissue. Deformities and reduced bone mass are the main bone manifestations in NF1. Quantitative computed tomography (QCT) provides true volumetric bone mineral density (BMD) measurement. This study aimed to evaluate bone metabolism parameters and BMD in children with NF1 using QCT.

Methods: The data of 52 paediatric NF1 patients (23 female, 29 male) was evaluated retrospectively. We investigated anthropometric measurements, biochemical parameters like total calcium, phosphate, magnesium, alkaline phosphatase, 25-hydroxyvitamin D (25OHD), parathyroid hormone, calcitonin, urinary calcium/creatinine ratio, and QCT parameters like lumbar trabecular and cortical BMD, trabecular area and cortical thickness. Comparisons of gender and puberty status were performed.

Results: 25% of patients had skeletal deformities and 42.3% had 25OHD inadequacy (<20 ng/mL). The frequency of 25OHD inadequacy was significantly higher in pubertal/postpubertal patients than prepubertal patients (61.9% vs. 29.0%, \( P = 0.019 \)). Trabecular BMD Z-score was \(<-2.0\) in 11.5% of patients; all with low BMD were at the pubertal/postpubertal stage. There was a significant negative correlation between age and trabecular Z-score (\( r = -0.41, P = 0.003 \)). Mean cortical BMD was statistically similar between the genders and puberty groups. Puberty status, anthropometric Z-scores, and biochemical and QCT parameters were statistically similar between the genders (\( P > 0.05 \)).

Conclusion: Paediatric NF1 patients may present with low BMD and 25OHD inadequacy, especially at puberty. QCT may be a useful tool to evaluate trabecular and cortical bone separately in NF1 patients.

Keywords: Bone mineral densitometry, children, neurofibromatosis, quantitative computed tomography

INTRODUCTION

Neurofibromatosis type 1 (NF1) is an autosomal dominant neurocutaneous disease characterised by café au lait spots, Lisch nodules, cutaneous neurofibromas, and a predisposition to benign and malignant tumours. NF1 has an incidence of one in 2,500 to 3,000 births and affects both genders equally.[1] The \( NF1 \) gene that is responsible for NF1 is located on the long arm of chromosome 17 at 17q11.2. It consists of 60 exons that are spread out over 350 kb of genomic DNA and encodes the protein, neurofibromin. Neurofibromin is expressed in many types of cells (including osteoblasts, osteoclasts and chondrocytes), regulates cell proliferation and differentiation, and acts as a tumour suppressor by inactivating the reticular activating system (RAS) signaling pathway. The RAS is the key regulator of several aspects of normal cell growth and malignant transformation.[2]

Clinical findings of NF1 include bone deformities. Its musculoskeletal findings include increased risk of fracture,
decreased bone mineral density (BMD), short stature and scoliosis or pseudoarthrosis of the long bones.\[13\] Osteoporosis with a frequency of 20%–50%, decreased serum 25-hydroxyvitamin D (25OHD) levels, increased serum parathyroid hormone (PTH) levels and increased serum bone turnover markers have been reported in adult patients with NF1.\[14\] Bone biopsies of NF1 patients showed an increase in osteoid volume and osteoclast/osteoblast counts.\[15\] The pathogenesis of the significant bone mass reduction in NF1 patients has not yet been clearly understood, but neurofibromin is thought to play a crucial role. As a result of deletions in the NF1 gene, neurofibromin loss occurs and intracellular RAS activity increases, leading to increased osteoclast activity and decreased osteoblast differentiation.\[6,7\]

In children with NF1, decreased BMD is a known fact.\[8\] Brunetti-Pierri et al.\[9\] and Caffarelli et al.\[10\] measured the lumbar vertebrae and femur of children with NF1, respectively, using dual-energy X-ray absorptiometry (DXA), with correction for height. Both studies concluded that children with NF1 have a lower BMD than healthy children.\[8,10\] In a Turkish paediatric NF1 study carried out using DXA to measure BMD, the ratios of patients with a Z-score <−2 at the lumbar vertebrae and femoral neck were found to be 21.2% and 9.1%, respectively.\[11\]

Quantitative computed tomography (QCT) is the only method that provides true volumetric BMD measurement (mg/cm³). QCT can analyse the cortical and trabecular bone separately, and it is more sensitive than DXA in monitoring bone loss. However, not enough research has been conducted to determine the reference QCT values in healthy children.\[12\] In the English literature, there has been no paediatric study evaluating BMD using QCT in NF1 patients. This study aimed to evaluate BMD using QCT and bone mineral metabolism in children with NF1.

**METHODS**

This observational study was carried out between October and December 2018. The local ethics committee approved the study (MEU 2018/316). The data of 52 patients who were diagnosed with NF1 according to the National Institutes of Health criteria\[13\] were retrospectively evaluated. The exclusion criteria were: endocrinopathy; delayed or precocious puberty; malignancy; hepatic and renal disorders; and inability to move or the use of a wheelchair.

Study data consisted of anthropometric measurements (height, weight, mid-arm circumference and triceps skinfold thickness), biochemical values (serum total calcium, phosphate, magnesium, alkaline phosphatase (ALP), PTH, 25OHD, calcitonin, vitamin B₁₂, folate, urine calcium, creatinine tests in local laboratory), and lumbar (L2–L4) spine QCT imaging obtained within the same day of outpatient visits in the last year. Body mass index (BMI, kg/m²), anthropometric Z-scores (according to the World Health Organisation charts) and urine calcium/creatinine ratio were measured. The threshold for serum 25OHD inadequacy was set at 25OHD <20 ng/mL.\[14\]

Lumbar spine trabecular area (cm²) and trabecular BMD (mg/cm²), cortical thickness (mm) and cortical BMD (mg/cm²) were the primary QCT parameters. An automatic low-dose QCT imaging protocol for paediatric patients was used (80 kV for 4–12 years, <100 kV for 12–16 years). Topograms for T11–L4 vertebrae were carried out using QCT (Alexion™, Toshiba Medical Systems, Tochigi, Japan) with Mindways QCT Pro 3D Spin BMD software (MindwaysInc, Austin, TX, USA). Analysis was performed on the L2–L4 vertebrae and all measurements were taken by the same radiologist. QCT reports, including BMD and Z-scores, were evaluated. Z-scores >−2.0 were accepted to be within the expected range, while Z-scores ≤−2.0 were defined as ‘low BMD-for-age’.\[15\]

There was no QCT data for the healthy control subjects due to ethical issues, and thus, group comparisons were made in terms of pubertal state and gender. Statistical analyses were conducted using IBM SPSS Statistics version 21.0 (IBM Corp, Armonk, NY, USA). For descriptive statistics, mean ± standard deviation (SD) or median (minimum–maximum) values were used for continuous variables, and numbers and percentages for categorical variables. Shapiro-Wilk test and histogram were used to test for normality. The independent groups were compared using Student’s t-test, Mann-Whitney U test or Chi-square test. Correlation analysis was performed to identify the relationships between age, QCT parameters and biochemical values. Statistical significance was set at P value <0.05.

**RESULTS**

A total of 52 NF1 patients aged 3–18 years were included in the evaluation. Of these, 55.8% were male. 59.6% of patients were at Tanner stage I (prepubertal), 32.7% at Tanner stage II–IV (pubertal), and 7.7% (n = 4) at Tanner stage V. The mean age of the patients was 9.8 ± 4.2 years. Pathological short stature (defined as height Z-score <−2) was found in 6 (11.5%) patients. 13 (25%) patients had skeletal abnormalities: mild scoliosis (n = 4); tibial bowing (n = 2); ulnar bowing (n = 1); genu varum (n = 3); ribbon rib deformity (n = 2); and femoral bone cyst (n = 1). None of the patients had a history of pathological fractures or vitamin D supplementation.

The overall mean values of biochemical parameters were within the normal range. Serum folate levels were normal in all patients, while 28 (53.8%) patients had a serum vitamin B₁₂ level below 200 pg/mL. PTH, calcitonin, total calcium, phosphate, magnesium and alkaline phosphatase concentrations were within the reference range. Urinary calcium/creatinine ratio was normal (≤0.2) in all the patients.
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In total, 22 (42.3%) patients (11 male, 11 female) had 25OHD <20 ng/mL, 3 (5.7%) patients had 25OHD <12 ng/mL, and 44 (84.6%) patients had 25OHD <30 ng/mL. 6 (11.5%) patients (3 male, 3 female) had a lumbar Z-score <−2. The characteristics and parameters are summarised in Table 1. There was no statistically significant difference between the male and female patients in terms of age, anthropometric Z-scores and biochemical values (P > 0.05). There was also no statistically significant difference between the genders in terms of pubertal state, frequency of low BMD, 25OHD inadequacy, and QCT parameters (P > 0.05).

There was no statistically significant difference between the prepubertal and pubertal/postpubertal groups in terms of gender and biochemical values (P > 0.05). The differences between the mean weight-for-age Z-scores and BMI-for-age Z-scores were statistically significant (P = 0.026 and P = 0.034, respectively). The height, weight and BMI-Z-scores were also within the range of (−1) to (+1) in both the prepubertal and pubertal/postpubertal groups. The frequency of 25OHD inadequacy was significantly higher in pubertal/postpubertal patients compared to prepubertal patients (P = 0.019). The pubertal/postpubertal group had a significantly lower mean trabecular BMD Z-score and a significantly higher mean trabecular area than the prepubertal group (P = 0.034 and P = 0.003, respectively). The frequency of low BMD was significantly higher in pubertal/postpubertal patients (P = 0.002). None of the prepubertal patient had low BMD. There was no statistically significant difference between the prepubertal and pubertal/postpubertal groups in terms of mean trabecular BMD, cortical BMD and cortical thickness (P > 0.05; Table 2).

No statistically significant difference was found between patients with and those without skeletal abnormalities in terms of anthropometric, biochemical and QCT parameters. Overall, age was significantly positively correlated with trabecular area, cortical thickness and PTH (r = 0.55, P < 0.01; r = 0.30, P = 0.030; r = 0.42, P = 0.002, respectively), and significantly negatively correlated with trabecular Z-score and phosphate (r = −0.41, P = 0.003; r = −0.35, P = 0.011, respectively). There were no significant correlations between magnesium, urinary calcium/creatinine ratio, vitamin B12, folate and QCT parameters (P > 0.05). Correlations between QCT parameters and calcium, phosphate, ALP, hormones are shown in Table 3. Anthropometric Z-scores were not significantly correlated with trabecular or cortical BMD (P > 0.05).

| Table 1. Gender comparisons in anthropometric, biochemical and densitometric data. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Characteristic/parameter                   | Overall (n=52) | Female (n=23) | Male (n=29)    | P                |
| Age (yr)                                | 9.8±4.2        | 10.4±4.4       | 9.3±4.1        | 0.373*           |
| Z-scores                                |                |                |                |                  |
| Height                                  | −0.53±1.25     | −0.55±1.19     | −0.52±1.31     | 0.944*           |
| Weight                                  | −0.65±1.15     | −0.41±1.08     | −0.85±1.18     | 0.176*           |
| Body mass index                         | −0.32±1.13     | −0.08±1.09     | −0.52±1.14     | 0.170*           |
| Mid-arm circumference                   | −1.46±1.26     | −1.21±1.29     | −1.66±1.23     | 0.198*           |
| Triceps skinfold thickness             | 0.05±0.72      | −0.14±0.71     | 0.21±0.69      | 0.077*           |
| Prepubertal stage                       | 59.6           | 47.8           | 69.0           | 0.123*           |
| Pubertal/Postpubertal stage             | 40.4           | 52.2           | 31.0           |                  |
| Total calcium (mg/dL) (vn 8.4-10.9)     | 9.7±0.3        | 9.8±0.3        | 9.7±0.4        | 0.493*           |
| Phosphate (mg/dL) (vn 3.3-5.6)          | 4.3±0.5        | 4.3±0.5        | 4.3±0.5        | 0.866*           |
| Magnesium (mg/dL) (vn 1.7-2.3)          | 2.03±0.13      | 2.01±0.10      | 2.05±0.14      | 0.303*           |
| Alkaline phosphatase (U/L) (vn 420-720)| 161 (44-242)   | 192 (44-242)   | 163 (58-309)   | 0.092*           |
| Parathyroid hormone (pg/dL) (vn 12-88) | 35.6±12.0      | 35.9±12.4      | 35.3±11.9      | 0.875*           |
| 25OHD (ng/mL) (vn>30)                   | 22.4±7.2       | 21.3±7.6       | 23.3±6.9       | 0.329*           |
| 25OHD inadequacy (<20 ng/mL)            | 42.3           | 47.8           | 37.9           | 0.473*           |
| Calcitonin (pg/mL) (vn 8-48)            | 4.3±3.8        | 3.1±1.8        | 5.2±6.3        | 0.278*           |
| Urine calcium/creatinine ratio (vn <0.2)| 0.10±0.06      | 0.09±0.05      | 0.11±0.06      | 0.314*           |
| Vitamin B12 (pg/mL) (vn 200-1,170)      | 210.0±76.9     | 205.1±69.0     | 213.9±83.7     | 0.686*           |
| Folate (mg/mL) (vn 3.8-16)              | 7.8±2.5        | 7.6±2.6        | 7.9±2.5        | 0.635*           |
| Trabecular BMD (mg/cm²)                 | 154.7±29.7     | 157.8±32.0     | 152.2±28.0     | 0.507*           |
| Trabecular Z-score                      | −0.71±1.27     | −0.72±1.31     | −0.70±1.26     | 0.936*           |
| Trabecular area (cm²)                   | 213.4±74.5     | 205.0±71.6     | 220.1±77.2     | 0.473*           |
| Cortical BMD (mg/cm²)                   | 257.1±47.1     | 271.4±48.8     | 245.9±43.1     | 0.051*           |
| Cortical thickness (mm)                 | 1.42±0.19      | 1.46±0.13      | 1.40±0.23      | 0.248*           |
| Low BMD (<−2 Z-score)                   | 11.5           | 13.0           | 10.3           | 1.00*            |

*Student’s t-test, data presented as mean±standard deviation. †Pearson Chi-square test, data presented as percentages. ‡Mann Whitney U test, data presented as median (min-max). §Fisher’s exact test, data presented as percentages. BMD: bone mineral density, 25OHD: 25-hydroxyvitamin D, vn: normal value.
DISCUSSION

NF1 is one of the most common single-gene diseases in humans. Low BMD, reduced bone mass, increased fracture risk and various skeletal deformities are significant complications of NF1. Previous studies have reported that in particular, adult NF1 patients have reduced BMD.\(^{[9]}\) BMD data belonging to paediatric NF1 patients has been growing in the recent decade. Impaired BMD has been detected in children with NF1 using DXA.\(^{[12,16-18]}\) In the present study, QCT was used to determine BMD status in paediatric NF1 patients, since QCT was the only option for evaluation of BMD in our centre.

Table 2. Comparison of anthropometric, biochemical and densitometric data by puberty status.

| Characteristic/parameter                  | Prepubertal (\(n=23\)) | Pubertal/postpubertal (\(n=29\)) | \(P\)  |
|------------------------------------------|-------------------------|----------------------------------|-------|
| Age (yr)                                 | 6.8±2.3                 | 14.2±2.1                         | <0.001* |
| Female                                   | 35.5                    | 57.1                             | 0.123† |
| Z-scores                                 |                         |                                  |       |
| Height                                   | −0.65±1.35              | −0.36±1.08                       | 0.424* |
| Weight                                   | −0.94±1.20              | −0.22±0.94                       | 0.026* |
| Body mass index                          | −0.60±1.09              | 0.07±1.09                        | 0.034* |
| Mid-arm circumference                    | −1.55±1.16              | −1.34±1.43                       | 0.560* |
| Triceps skinfold thickness               | 0.13±0.72               | −0.06±0.72                       | 0.326* |
| Total calcium (mg/dL) (vn 8.4-10.9)      | 9.7±0.2                 | 9.7±0.5                          | 0.892* |
| Phosphate (mg/dL) (vn 3.3-5.6)           | 4.4±0.4                 | 4.1±0.5                          | 0.059* |
| Magnesium (mg/dL) (vn 1.7-2.3)           | 2.04±0.14               | 2.01±0.10                        | 0.449* |
| Alkaline phosphatase (U/L) (vn<720)      | 162 (70-309)            | 151 (44-294)                     | 0.608† |
| Parathyroid hormone (pg/dL) (vn 12-88)   | 32.9±9.7                | 39.5±14.2                        | 0.074* |
| 25OHD (ng/mL) (vn>30)                    | 23.7±6.1                | 20.5±8.4                         | 0.125* |
| 25OHD inadequacy (<20 ng/mL)             | 29.0                    | 61.9                             | 0.019† |
| 12-20 ng/mL                              | 29.0                    | 47.6                             |       |
| Calcium (mg/g/L) (vn 0-8.4)              | 3.7±2.4                 | 5.4±7.6                          | 0.389* |
| Urine calcium/creatinine ratio (vn<0.2)  | 0.11±0.06               | 0.09±0.05                        | 0.257* |
| Vitamin B₁₂ (pg/mL) (vn 200-1,170)       | 219.0±80.3              | 196.7±71.4                       | 0.312* |
| Folate (ng/mL) (vn 3.8-16)               | 8.2±2.5                 | 7.0±2.4                          | 0.099* |
| Trabecular BMD (mg/cm³)                  | 155.5±26.6              | 153.4±34.4                       | 0.813* |
| Trabecular Z-score                       | −0.40±1.09              | −1.16±1.40                       | 0.034* |
| Trabecular area (cm²)                    | 186.8±57.9              | 252.7±80.0                       | 0.003* |
| Cortical BMD (mg/cm³)                    | 254.2±48.2              | 261.6±46.1                       | 0.582* |
| Cortical thickness (mm)                  | 1.38±0.21               | 1.49±0.16                        | 0.056* |
| Low BMD (<-2 Z-score)                    | 0                       | 28.6                             | 0.002‡ |

*Student’s t-test, data presented as mean±standard deviation. †Pearson Chi-square test, data presented as percentages. ¶Mann Whitney \(U\) test, data presented as median (min-max). ‡Fisher’s exact test, data presented as percentages. BMD: bone mineral density, 25OHD: 25-hydroxyvitamin D, vn: normal value.

Table 3. Correlations between quantitative computed tomography and biochemical parameters.

|                  | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trabecular area  | -   |     |     |     |     |     |     |     |     |     |
| Trabecular BMD   | −0.28* | -   |     |     |     |     |     |     |     |     |
| Trabecular Z-score | −0.41† | 0.93† | -   |     |     |     |     |     |     |     |
| Cortical BMD     | −0.24 | 0.54† | 0.43† | -   |     |     |     |     |     |     |
| Cortical thickness | 0.09 | 0.06 | −0.03 | 0.19 | -   |     |     |     |     |     |
| Parathyroid hormone | 0.37† | −0.15 | −0.32* | −0.06 | 0.10 | -   |     |     |     |     |
| Calcitonin       | −0.09 | −0.15 | −0.26 | −0.04 | 0.22 | 0.20 | -   |     |     |     |
| 25OHD            | 0.02 | 0.25 | 0.33* | 0.10 | 0.09 | −0.20 | −0.21 | -   |     |     |
| Total calcium    | 0.04 | 0.14 | 0.10 | 0.26 | −0.02 | −0.05 | −0.28 | 0.11 | -   |     |
| Phosphate        | 0.07 | 0.13 | 0.22 | 0.00 | 0.10 | −0.02 | −0.59† | 0.31* | 0.01 | -   |
| Alkaline phosphatase | 0.12 | −0.14 | −0.08 | −0.11 | −0.08 | −0.00 | −0.20 | 0.07 | 0.10 | 0.23 |

*\(P<0.05\), †\(P<0.01\) (2-tailed); Pearson’s correlation test. BMD: bone mineral density, 25OHD: 25-hydroxyvitamin D.
QCT is superior in that it provides a tridimensional imaging of the bone and analyses the trabecular and cortical bones separately. Also, QCT can measure volumetric bone mass independently of bone size. Trabecular and cortical bone structure and geometry can also be evaluated with relatively low radiation exposure (0.59–1.09 mSv) in children. Unlike DXA, QCT values are not influenced by in homogeneities in soft tissue composition and fat distribution; hence, QCT is not limited by growth-related variations in bone, body size and body composition.

To the best of our knowledge, this is the first study to evaluate BMD using QCT in paediatric NF1 patients. The trabecular bone is eight times more metabolically active than the cortical bone, and trabecular bone density (TBD) in the vertebral body is a valuable parameter in the assessment of age-related bone loss. In the present study, it was found that pubertal/postpubertal NF1 patients had significantly lower TBD Z-scores than prepubertal patients. We also found that low TBD was a complication for only pubertal/postpubertal patients and the TBD Z-score was negatively correlated with age. However, it is known that pubertal development and age have positive influences on volumetric BMD in adolescence, and humans achieve peak bone mass by the end of pubertal development. Our findings show that the trabecular area increases with age as an indication of axial skeleton growth but bone mineralisation does not, which is worrisome in terms of bone accumulation in paediatric NF1 patients during puberty. Similarly, Rodari et al. suggested progressive bone mineralisation impairment with age and pubertal development in NF1 patients with a mean age of 11.6 ± 4 years. So, interventions to optimise bone accumulation in paediatric NF1 patients appear to be essential to prevent NF1-related osteoporosis. In a study by Poyrazoğlu et al., the femur BMD and femur Z-score were significantly positively correlated. Likewise, lumbar TBD and lumbar Z-score were strongly correlated in our study.

Since the 1990s, it has been known that the cortical area and the density of the cortical bone increase with skeletal maturation in peri-adolescents. The vertebral body has a thin and porous cortical shell. Cortex gains surface area but becomes more porous and loses thickness with ageing and diseases. In the present study, there was a mild positive correlation between age and cortical thickness; contrary to expectations, cortical thickness or cortical BMD did not significantly increase with puberty. These findings suggest that cortical bone thickening and mineralisation impairments can occur in NF1 patients during adolescence, and that starting from adolescence, vertebral fragility can become a complication. A moderate positive correlation between cortical and trabecular BMD indicates that paediatric NF1 patients lose BMD in both the cortical and trabecular compartments.

In the present study, phosphate showed a negative correlation with age and calcitonin, and a positive correlation with 25OHD. Phosphate is essential for bone mineralisation, and our findings confirm that infants have the highest serum phosphate concentration in general, and that serum phosphate declines towards adulthood. Calcitonin has a phosphaturic effect, while vitamin D is one of the physiological regulators of intestinal phosphate absorption. We suggest that phosphate haemostasis in NF1 patients is similar to that of the healthy subjects. The relation between serum PTH and BMD has been found to be related to the type of bone measured, and serum PTH was reported to be negatively associated or non-associated with BMD after adjustment for lifestyle factors in several osteoporosis studies. Our results indicate a negative correlation between PTH and BMD Z-score in paediatric NF1 patients.

The frequency of 25OHD deficiency or insufficiency is higher in children with NF1 than in general population. Calcium absorption abnormalities, inadequate sun exposure, preference for clothing that covers exposed skin due to cutaneous manifestations and limited outdoor activities because of several systemic involvements may contribute to vitamin D inadequacy in NF1 patients. In a 2018 report, Rodari et al. revealed that there was a high prevalence of 25OHD insufficiency in NF1 patients (60% with <20 ng/mL, 98% with <30 ng/mL). A study from Turkey found that 63.6% of the 33 NF1 paediatric patients had a 25OHD level of ≤20 ng/mL and 90.9% had a 25OHD level of ≤30 ng/mL. Our results confirm these previous findings. However, Poyrazoğlu et al. determined that the 25OHD level was significantly lower in female vs. males patients aged ≥8 years. In the present study, the 25OHD level and the frequency of 25OHD deficiency were similar in girls and boys, but the frequency of 25OHD inadequacy was significantly higher in pubertal/postpubertal patients.

The present study did not examine other factors that may influence vitamin D levels, such as physical activity, daily diet, sunlight exposure, geographical region, weather and seasonal changes. Our results also differed from those of previous studies in that we identified a significant correlation between 25OHD and trabecular Z-score. Stevenson et al. reported that 25OHD was not associated with subtotal body BMD Z-scores, although there was a general trend of a positive correlation between 25OHD levels within the deficient range and BMD Z-scores. Potential explanations for the difference in findings include factors such as race, geographical location, lifestyle, and NF1-related cutaneous and skeletal manifestations. Further studies with a larger sample size, which include NF1 individuals with 25OHD deficiency and insufficiency, should investigate the association between low 25OHD levels and the degree of impaired BMD. Daily calcium (1,200 mg) and vitamin D (800 IU) supplementation for 12 months has been found to be effective for restoring hypovitaminosis D but not for low BMD in adults. The etiopathogenesis of a high prevalence of hypovitaminosis D, its association with bone growth failure and therapeutic approaches in paediatric NF1 patients are investigable issues.
Adult studies reported that low vitamin B₁₂ and folate levels were related to lower BMD. Vitamin B₁₂ and folate deficiencies are associated with high serum homocysteine, and high homocysteine concentration is associated with increased fracture risk. Changes in collagen cross-linking and bone structure are the potential pathogenetic mechanisms.²⁹ It is known that NF1-related osteopenia progresses to osteoporosis since BMD decreases with ageing.³⁰ In the present study, even though vitamin B₁₂ and folate levels were not associated with trabecular or cortical BMD, the paediatric NF1 patients should be followed up for hypovitaminosis, as more than half of the patients had a serum vitamin B₁₂ level below 200 pg/mL.

The frequency of skeletal abnormality was reported to be within the range of 18.2%–33% in paediatric NF1 studies from Turkey, with scoliosis found to be the most common deformity,³¹,³₂,³³ and this is in accordance with our results. Duman et al. suggested that osteocalcin, and not DXA findings, was the predictor of skeletal abnormalities in NF1 patients.³¹ In the present study, no predictive analysis was performed, and bone resorption markers were not evaluated. We evaluated only ALP as a bone formation marker and did not find any association between ALP and BMD. In our study, as in previous studies, reduced bone mass was found to be a complication for NF1 patients, both with and without deformity.⁴,²⁸,³³

The limitations of the present study included: not having a control group; taking our laboratory normal values as reference; including patients from different geographical regions and data from different seasons; not evaluating dietary and physical activity factors; a small sample size; and evaluating only ALP as a bone turnover marker. Despite the limitations, the strength of the present study was that it presented data about trabecular and cortical BMD separately using QCT. Our findings show that NF1 patients lose BMD in both the cortical and trabecular compartments and that low BMD emerges with puberty. In future, there is a need for larger-size seminal studies that evaluate osteoblastic and osteoclastic activity markers in paediatric NF1 patients using QCT measurements.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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