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ORIGINAL ARTICLE

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Bone mineral density in children with familial Mediterranean fever

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Abstract The aim of this study was to evaluate bone mineral content (BMC), serum and urinary bone turnover parameters in patients with familial Mediterranean fever (FMF), an autosomal recessive disease characterized by recurrent episodes of inflammation of serous membranes. Demographic characteristics and MEFV mutations were defined in 48 children diagnosed with FMF (23 F, 25 M; median age 7.0 years (3.0–10.0)). We evaluated the blood counts, acute-phase proteins and serum and urinary bone turnover parameters during attack-free periods. The BMC and BA (bone area) of vertebrae L1–L4 were measured by DEXA. Thirty-eight age-, sex- and ethnicity-matched healthy children constituted the control group. Mean L1–L4 BMC in Group I (patients with two mutations) and II (patients with no or single mutations) were 15.49 ± 5.99 g and 15.68 ± 4.89 g, respectively, both significantly lower than the mean L1–L4 BMC of control patients, which was 19.59 ± 6.7 g ($p < 0.05$). Mean L1–L4 BMD in Group I, Group II and the control group were 0.466 ± 0.066 g/cm^2, 0.487 ± 0.085 g/cm^2 and 0.513 ± 0.079 g/cm^2, respectively. Mean z-scores in Group I, Group II and the control group were -1.87 ± 0.74, -1.55 ± 0.92 and -1.39 ± 0.84, respectively. Mean L1–L4 BMD and z-score of Group I were lower than in the control group ($p < 0.05$). ESR and SAA (serum amyloid A) levels were higher in Group I patients: 28.3 ± 14.5 mm/h and 350 ± 62 mg/l in Group I; and 20.5 ± 11.7 mm/h and 190 ± 68 mg/l in Group II, respectively. In conclusion, FMF patients had lower BMC, BMD and z-scores than a control group. We suggest that decreased BMD, BMC and z-score in FMF patients may be secondary to subclinical inflammation.

Keywords Bone mineral density · DEXA (dual energy X-ray absorptiometry) · Familial Mediterranean fever · z-score

Abbreviations BA: Bone area · BMC: Bone mineral content · BMD: Bone mineral density · bsALP: Bone-specific alkaline phosphatase · BMI: Body mass index · DEXA: Dual energy X-ray absorptiometry · DGGE: Denaturing gradient gel electrophoresis · DPD/Cre: Deoxypyridinoline/creatinine · FMF: Familial Mediterranean fever · SAA: Serum amyloid A

Introduction

Familial Mediterranean fever (FMF), which primarily affects Sephardic Jews, Armenians, Turks and North African Arabs, is a recessively inherited disorder. The diagnosis is based on the occurrence of recurrent episodes of fever and serosal inflammation, manifested by sterile peritonitis, arthritis, and pleurisy. Amyloidosis of AA type, mainly renal, is the major complication of FMF [1]. Regular colchicine use both prevents amyloidosis and decreases the frequency, duration and intensity of FMF attacks. The gene responsible for FMF, designated MEFV, on chromosome 16 (16p13.3) was identified in 1997 by two independent groups (French FMF Consortium and International FMF Consortium) [2, 3]. The number of defined mutations is about 30.

Recent studies have shown that subclinical inflammation may continue in some cases of FMF, even in symptom-free patients [4, 5]. Subclinical inflammation might have numerous side effects, especially in children: loss of appetite, and impairment of physical and mental development. The long-term effects of subclinical inflammation in FMF are not well evaluated. Zemer et al. analysed the compulsory medical examination

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prior to military service, at age 17 [6]. They showed that the mean height of the colchicine-treated cohort with FMF had increased compared to the cohort of FMF patients who had not received colchicine.

There is an increasing clinical interest in measurement of bone mineral content (BMC) in children, especially in the evaluation of adverse effects of disease. Loss of appetite, decreased physical activity during attacks and continuous subclinical inflammation may result in decreased bone mineral content in children with FMF.

Dual energy X-ray absorptiometry (DEXA) is a non-invasive technique for the assessment of BMC and bone mineral density (BMD). BMD is expressed conventionally as ratio of measured BMC and the projected bone area (BA), i.e. BMC/BA.

The aim of this study was to measure BMC, BMD, z-score, serum and urinary bone turnover parameters, and to evaluate the influence of demographic and genetic factors on these parameters in prepubertal children with FMF.

Patients and methods

In this cross-sectional study we evaluated 48 prepubertal children with FMF [23 female, 25 male; median age 7.0 years (3.0–10.0)]. The diagnosis of FMF was established according to previously described criteria [7]. The clinical data were registered on a standardized form that included age, gender, familial consanguinity, history of the disease, age at onset of symptom(s), age at diagnosis, presence of fever, abdominal pain, pleurisy, joint pain and other features. Height, weight and bone age were measured, and body mass index (BMI: weight/stature squared, in kg/m^2) and standard deviation scores (SDS) for height and BMI were calculated. SDS were calculated using the formula: (measurement [height or BMI]-mean [height or BMI] for respective sex and age)/standard deviation [height or BMI] with respect to age and sex. British standards were used to calculate SDS [8, 9]. All patients were on colchicine therapy. Informed consent was obtained from the patient or the parents.

We evaluated the blood counts, erythrocyte sedimentation rate (ESR; normal <20 mm/h), C-reactive protein (CRP, normal <800 μg/l), serum amyloid A (SAA, normal: <10 mg/l), serum total protein (normal: 61–79 g/l for 3–7 years of age, 64–81 g/l for 8–10 years of age) and albumin (normal: 39–50 g/l for less than 5 years of age, 40–53 g/l for more than 5 years of age), serum total calcium (normal: 2.2–2.7 mmol/l), phosphorus (normal: 1.2–1.8 mmol/l), bone-specific alkaline phosphatase (bsALP) (normal: 63–139 U/l), parathyroid hormone (PTH) (normal: 12–72 ng/ml), osteocalcin (normal: 51–84 μg/l), serum carboxy-terminal type 1 collagen telopeptide (ICTP) (normal: 147–558 μg/l), vitamin D (normal: 25–100 nmol/l), and urinary deoxypyridinoline/creatinine (DPD/Cre) ratio (normal: 22.4 ± 1.4 nmol/mmol). Serum parathyroid hormone, bone specific alkaline phosphatase, osteocalcin, carboxy-terminal propeptide of type I procollagen, and urinary deoxypyridinoline were measured by immunoassay using commercial kits (Metra Biosystems, Mountain View, CA, USA). Blood and urine samples were drawn during attack-free period (at least 14 days after an FMF attack). Any patient with a disease other than FMF was excluded.

Mutation analysis

M964V, M680I, V726A, M964I, E148Q are the main mutations in FMF patients in the Turkish population [10]. Our strategy for mutation analysis includes two steps. The hot spot exon 10, which harbors 14 mutations, is first analysed by denaturing gradient gel electrophoresis (DGGE). According to the band pattern, subsequent analysis is done either by restriction endonuclease enzyme digestion or by sequencing. Furthermore, E148Q in exon 2 is analysed by restriction endonuclease enzyme digestion.

The patients were divided into groups according to their mutation analysis: Group I, patients with two mutations, and Group II, patients with single or no mutations.

Control group

L1–L4 BMC, BMD and z-scores were measured in 38 healthy children, all in the prepubertal period and matched for age, sex and ethnicity. Demographic data and laboratory values were compared between the groups.

Measurement of BMC and BA

BMC and BA (bone area) of vertebrae L1–L4 were measured by dual energy X-ray (Hologic QDR-4500A). Normative data for BMD in Turkish children are not available, and so z-scores for BMD were determined by comparing measurements with normal data from the manufacturer.

Statistical analysis

Student's t-test, and the χ^2test were used for comparison. A p value of <0.05 was considered to indicate statistical significance. Data were expressed in mean $\pm$ SD (standard deviation).

Results

Demographic characteristics and MEFV mutation analysis of Group I (13 female, 14 male, mean age 6.75 ± 2.42 years) and Group II patients (10 female, 11

female, mean age 6.71 ± 2.22 years) are summarized in Table 1. Fever (95%–100%) and abdominal pain (95%–100%) were the leading symptoms, followed by arthritis/arthralgia (70%–75%) and chest pain (37%–38%); two groups were comparable in terms of demographic characteristics. Twenty-seven patients were homozygous or compound heterozygous, M694V/M694V genotype being the most common genotype; 10 patients had a single mutation, and 11 patients were negative for M964V, M680I, V726A, M964I and E148Q mutations.

Anthropometric measurements of patients with FMF were compared to those of controls (Table 2). Mean height, weight and BMI, as well as bone age, were slightly greater in the control group than in either group of patients; however, none of these parameters reached statistical significance when compared among the three groups. Furthermore, height and BMI standard deviation scores (SDS) were similar in the three groups studied ($p = 0.169$, and $p = 0.612$, respectively) (Table 2).

Mean L1-L4 BMC in Group I and II were 15.49 ± 5.99 g and 15.68 ± 4.89 g, respectively, both significantly lower than the mean L1–L4 BMC of control patients: 19.59 ± 6.7 g ($p < 0.05$). Mean L1–L4 BMD in Group I, Group II and the control group were 0.466 ± 0.066 g/cm^2, 0.487 ± 0.085 g/cm^2 and 0.513 ± 0.079 g/cm^2, respectively. Mean z-scores in Group I, Group II and the control group were –1.87 ± 0.74, -1.55 ± 0.92 and –1.39 ±

Table 1 Demographic characteristics and MEFV mutation analysis of 48 patients with FMF

Features	Group I (2 mutations) ($n = 27$)	Group II (no or single mutation) ($n = 21$)	p
Gender (female/male) (n/n)	13/14	10/11	NS
Current age (years, mean ± SD)	6.71 ± 2.22	6.75 ± 2.42	NS
Age at onset of symptoms (years, mean ± SD)	2.75 ± 2.06	3.04 ± 1.74	NS
Consanguinity n (%)	4 (16.7)	4 (16.7)	NS
Family history n (%)	11 (45.8)	9 (37.5)	NS
History of appendectomy n (%)	2 (8.3)	0 (–)	NS
Frequency of attacks before diagnosis (attack/year; mean ± SD)	19.8 ± 15.8	22.9 ± 16.2	NS
Duration of attacks before diagnosis (days; mean ± SD)	2.3 ± 2.9	2.0 ± 1.4	NS
Frequency of attacks after colchicine (attack/year; mean ± SD)	1.6 ± 2.8	1.4 ± 1.9	NS
Duration of attacks after colchicine (days; mean ± SD)	0.6 ± 0.9	0.4 ± 0.4	NS
Clinical features			
Fever n (%)	27 (100.0)	20 (95.2)	NS
Abdominal pain n (%)	27 (100.0)	20 (95.2)	NS
Chest pain n (%)	10 (37.0)	8 (38.1)	NS
Arthritis/arthralgia n (%)	19 (70.4)	16 (76.2)	NS
MEFV mutations			
M694V/M694V	14	–	
M694V/M680I	6	–	
M694V/V726A	5	–	
M680I/M680I	1	–	
V726A/E148Q	1	–	
M694V/–	–	7	
M680I/–	–	1	
V726A/–	–	1	
E148Q/–	–	1	
Unidentified/unidentified	–	11	

Table 2 Bone mineral content (BMC), bone mineral density (BMD) and z-scores of study groups

Features	Group I (2 mutations) ($n = 27$)	Group II (No or single mutation) ($n = 21$)	Control group ($n = 38$)	p
Female/male	13/14	10/11	20/18	NS
Age (years)	6.71 ± 2.22	6.75 ± 2.42	6.68 ± 2.73	NS
Height (cm)	119.9 ± 12.25	121.38 ± 14.86	124.11 ± 17.36	NS
Weight (kg)	22.2 ± 6.1	24.8 ± 10.0	26.0 ± 9.18	NS
BMI (kg/m^2)	15.93 ± 2.00	16.16 ± 2.94	16.5 ± 2.63	NS
HSDS	0.06 ± 1.08	0.55 ± 1.02	0.44 ± 1.33	NS
BMI SDS	0.09 ± 1.13	–0.01 ± 1.31	0.21 ± 1.45	NS
Bone age (years)	6.38 ± 2.40	6.50 ± 2.31	6.90 ± 2.73	NS
L1–L4 BMC (g)	15.49 ± 5.99	15.68 ± 4.89	19.59 ± 6.7	$< 0.05^{a,b}$
L1–L4 BMD (g/cm^2)	0.466 ± 0.066	0.487 ± 0.085	0.513 ± 0.079	$< 0.05^{a}$
z score	–1.87 ± 0.74	–1.55 ± 0.92	–1.39 ± 0.84	$< 0.05^{a}$

BMI: body mass index; BMI SDS: BMI standard deviation score; HSDS: height standard deviation score

[a]Comparison between control group and Group I

[b]Comparison between control group and Group II

Comparison between Group I and Group II was not significant ($p > 0.05$)

0.84, respectively. Mean L1–L4 BMD and z-score of Group I were lower than in the control group ($p < 0.05$). The mean values in Group II were higher than in Group I, and lower than in the control group, but these differences did not reach statistical significance (Table 2).

Comparison of acute phase proteins, and serum and urinary bone turnover parameters of patients revealed (Table 3) that mean haemoglobin, haematocrit, calcium, phosphorus, bsALP and ICTP were within normal limits, and comparable in Group I and II patients. Vitamin D levels were in the lower range. In Group I, 13 patients had vitamin D levels < 25 nmol/l, and 4 other patients had levels between 25 and 40 nmol/l. The remaining patients had vitamin D > 40 nmol/l. In Group II 7 patients had vitamin D < 25 nmol/l, 8 had levels between 25 and 40 nmol/l, and the remaining had levels > 40 nmol/l. Osteocalcin was in the lower limit of normal in Group II, and even lower in Group I patients; however, the difference between the two groups did not reach statistical significance. DPD/Cre was slightly elevated in both groups of patients. ESR and SAA levels were higher in Group I: 28.3 ± 14.5 mm/h and 350 ± 62 mg/l in Group I and 20.5 ± 11.7 mm/h and 190 ± 68 mg/l in Group II, respectively ($p < 0.05$).

Discussion

To the best of our knowledge this is the first study evaluating bone mineral density in patients with FMF. In this cross-sectional study we showed that FMF patients with mutations in both alleles had significantly lower BMC, BMD and z-score values than the control group.

The difference in BMC and BMD of patients with FMF compared to controls cannot be explained by differences in body size or skeletal maturation, as height and BMI SDS as well as bone age were found to be statistically similar in patients and controls. We have observed low z-scores not only in patients but also in controls. This may be due to the method of determination, for we used normative data from the manufacturer, as normative values for BMD in Turkish children are not available. However, all three groups were subject to the same method of evaluation, and we would expect the bias caused by the method of determination of z-scores to affect the measurements in patients and controls alike, as the patients and controls were comparable not only with respect to age but also in their body size.

Biochemical markers of bone turnover did not deviate significantly from normal in the patients. DPD/Cre, which is a marker for bone resorption, was slightly over the upper limit of normal in both patient groups. This may suggest increased resorption in these patients. Vitamin D levels too were in the lower range. It may be speculated that less than optimal levels of vitamin D in these patients may lead to bone resorption, for it is well known that vitamin D is essential for the maintenance of adequate calcium levels for bone mineralization. Levels above 40–50 nmol/l are suggested to be sufficient for vitamin D status; however, the optimal level of serum 25-OH-D necessary for promotion of bone mineral accretion during growth has not been fully established in children. Two studies investigated the impact of vitamin D deficiency during the winter season on bone mass in children and young adults [11, 12]. Neither demonstrated a correlation between serum 25-hydroxy vitamin D and DXA BMD in children and adolescents. This may be due to the fact that bone mineral accretion is cumulative, and vitamin D levels at a single point may not correlate well with a single bone mass measurement. PTH levels may provide and index of the physiological significance of low vitamin D status, for it indicates suboptimal calcium status before decreases in the serum calcium level occur. Normal levels of PTH in the current cohort preclude vitamin D deficiency as a significant determinant of bone mineral status.

Another possibility is that ongoing inflammation in these patients, as suggested by elevated ESR in Group I, may be a cause for increased bone resorption.

Osteocalcin levels were below the normal range in the patients, more so in Group I. Osteocalcin is a marker for bone formation, and low levels in patients with FMF may suggest suppression of bone formation as a factor leading to decreased mineralization. However, we did

Table 3 Comparison of clinical and laboratory features according to mutation analysis

Laboratory tests	Group I (2 mutations)	Group II (no or single mutation)	p
Haemoglobin (mmol/l)	1.85 ± 0.16	2.01 ± 0.16	NS
Haematocrit (%)	36.1 ± 2.7	36.4 ± 2.8	NS
Total protein (g/l)	64.7 ± 7.1	68.4 ± 7.7	NS
Albumin (g/l)	43.2 ± 4.5	45.3 ± 0 4.8	NS
ESR (mm/h)	28.3 ± 14.5	20.5 ± 11.7	< 0.05
CRP (μg/l)	560 ± 700	480 ± 470	NS
SAA (mg/l)	350 ± 62	190 ± 68	< 0.05
Calcium (mmol/l)	2.5 ± 0.2	2.5 ± 0.1	NS
Phosphorus (mmol/l)	1.65 ± 0.19	1.58 ± 0.16	NS
DPD/Cre (nmol/mmol)	30.6 ± 14.7	25.2 ± 8.3	NS
Bone specific ALP (U/l)	85 ± 41	83 ± 30	NS
Osteocalcin (μg/l)	12.3 ± 9.8	20.5 ± 40.1	NS
ICTP (μg/l)	399 ± 111	317 ± 137	NS
Vitamin D (nmol/l)	29.7 ± 17.5	30.9 ± 15.5	NS
PTH (ng/ml)	50.16 ± 30.00	43.01 ± 18.67	NS

ALP: alkaline phosphatase; bsALP: bone specific alkaline phosphatase; CRP: C-reactive protein; DPD/Cre: urinary deoxypyridinoline/creatinine ratio; ESR: erythrocyte sedimentation rate; ICTP: serum carboxy-terminal type 1 collagen telopeptide; SAA: serum amyloid A

not observe a similar trend in other markers of bone formation, i.e. bsALP and ICTP. Osteocalcin is labile, and measurements are subject to errors arising from sample processing and storage. On the other hand ICTP and bsALP are much more stable than osteocalcin, and hence less sensitive to processing and storage conditions. The low levels of osteocalcin in the face of normal levels of bsALP and ICTP may simply arise from the difficulties of determining osteocalcin in serum.

Lack of data in controls is the main constraint of this study, however, and it is difficult to draw any conclusions from the current study as to the specific aetiology of decreased bone mineral density in patients with FMF. Factors that could have an impact on bone mineralization, such as age at diagnosis, sex, frequency and duration of the attacks, and levels of SAA, were analysed using multivariate analysis, but this was inconclusive.

One who is not familiar with FMF might think that some patients with single or no mutations were overdiagnosed. There are about 30 mutations defined in FMF, and it is time-consuming and expensive to study all of them. The diagnosis is based on clinical criteria rather than MEFV gene mutation analysis [7]. Like most FMF centres, we studied the most common mutations in our population: M694V, M680I, V726A, M694I and E148Q [10, 13, 14]. Furthermore, the patients with single or no mutations had begun to suffer FMF attacks once their colchicine therapy was interrupted. So there was no doubt about the diagnosis.

Patients with two mutations (Group I) had higher ESR and SAA levels. We had previously shown that FMF patients who were homozygous or compound heterozygous for most common mutations (M694V, M680I, V726A, M694I, E148Q in our population) had a higher level of subclinical inflammation than those who had no or single mutations [5]. We suggest that decreased BMD, BMC and z-score in patients with two mutations (Group I) were secondary to increased subclinical inflammation. We think that the difference between Groups I and II and Group II and controls may reach statistical significance in a larger study group.

In order to determine the exact role of colchicine on BMD in children with FMF, prospective case–control studies comparing groups with and without colchicine therapy are necessary, but it is unethical not to institute colchicine once the diagnosis is made.

Longitudinal studies may give answer to following questions:

- Do BMD and z-score improve as the child with FMF grows up?
- Does a better control of subclinical inflammation in patients with FMF ameliorate BMC, BMD and z-score? In other words, do patients with consistently low ESR or SAA level have increased BMC, BMD and z-score than patients with consistently high ESR or SAA levels?

In summary, we have shown that FMF patients had lower BMC, BMD and z-scores than a control group matched for age, sex and ethnicity. We suggest that decreased BMD, BMC and z-score in FMF patients may be secondary to subclinical inflammation. Early diagnosis and regular colchicine will not only decrease the intensity of FMF attacks and prevent renal amyloidosis, may but also contribute to a better mineralization of bone.

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