Original Article

Bone Mineral Density in Egyptian Children with Familial Mediterranean Fever

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What's Known

• In the past, diagnosis of FMF was based on clinical manifestations, ethnicity, family history, and response to colchicine treatment. In cases where these components are atypical or unhelpful, FMF was difficult to diagnose.
• After identification and isolation of the gene responsible for FMF (MEFV) on the short arm of chromosome 16, diagnosis of FMF became more reliable.
• FMF patients had significantly lower BMD values than the control group.

What's New

• There are predominantly four gene mutations among Egyptian children: E148Q, V726A, M680I and M694V.
• There were four heterozygous gene mutations in 40% of the control children.
• Molecular genetic analysis could be used for early and correct diagnosis of FMF in children with suspected manifestations.
• Regular use of colchicine treatment improves the effect of FMF and BMD, mainly at the femur and up to the normal values.

Abstract

Background: Familial Mediterranean fever (FMF) has episodic or subclinical inflammation that may lead to a decrease in bone mineral density (BMD). The objective of this study was to assess BMD in Egyptian children with FMF on genetic basis.

Methods: A cross sectional study included 45 FMF patients and 25 control children of both sexes in the age range between 3-16 years old. The patients were reclassified into two groups, namely group I(A) with 23 cases using colchicine for 1 month or less, and group I(B) with 22 cases using colchicine for more than 6 months. For both the patients and control groups, MEFV mutations were defined using molecular genetics technique and BMD was measured by DXA at the proximal femur and lumbar spines.

Results: Four frequent gene mutations were found in the patient group E148Q (35.6%), V726A (33.3%), M680I (28.9%), and M694V (2.2%). There were also four heterozygous gene mutations in 40% of the control children. Patients receiving colchicine treatment for less than 1 month had highly significant lower values of BMD at the femur and lumbar spines than the control children (P=0.007, P<0.001). Patients receiving colchicine treatment for more than 6 months had improved values of BMD at femur compared with the control, but there were still significant differences between them in lumbar spine (P=0.036). There were insignificant effect of gene mutation type on BMD and the risk of osteopenia among the patients.

Conclusion: FMF had a significant effect on BMD. However, regular use of colchicine treatment improves this effect mainly at the femur.

Introduction

Familial Mediterranean fever (FMF) is a hereditary disease (an autosomal recessive).[1] The attack frequency differs from one patient to another. Patients are symptom free between the attacks, although some have ongoing subclinical inflammation without symptoms.[2] The main treatment for FMF is colchicine as it reduces attack frequency and duration in most patients.[3]

In the past, diagnosis of FMF was based on clinical manifestations, ethnicity, family history, and response to colchicine treatment. In cases where these components are atypical or unhelpful, FMF is difficult to diagnose. After identification and

isolation of the gene responsible for FMF (MEFV) on the short arm of chromosome 16, diagnosis of FMF became more reliable.[4]

The MEFV gene was independently cloned by American and French groups in 1997. The protein encoded by MEFV gene has been named pyrin for its role in anti-pyrexia. It normally regulates inflammation via apoptotic speck-like protein. In FMF, however, the pyrin derived from the mutated gene seems to lose the ability to regulate the normal inflammatory process.[5]

To date, 142 mutations have been identified in the MEFV gene, most of which are substitutions (78 of them are missense, one nonsense, 39 silent mutations, 17 are located in introns, two in UTS), one is duplication, two are insertions and two are deletions. Of these mutations, five accounts for more than 70% of FMF cases (V726A, M694V, M694I, M680I, and E148Q) and have different frequencies in classically affected populations.[6] Exons 2 and 10 are the most frequent mutation regions of the MEFV gene. Half of the FMF population carries two mutations while 30% and 20% carry a single mutation and no identifiable mutation, respectively.[7]

Heterozygous patients should have more strong clinical evidence regarding FMF because they can actually have FMF or merely be carriers. By definition, anyone with two copies of MEFV mutations is considered to have FMF. The situation is more complex for individuals with one MEFV mutation.[8]

Children may be subject to both primary and secondary bone loss. Primary bone loss involves genetic mutations leading to defects in either collagen synthesis or in conservation of bone mineral. Secondary bone loss results from the body response to a variety of acute and chronic conditions.[9]

Osteoporosis and osteopenia are generalized disorders of the skeleton where the reduced bone quality and low bone density results in decreased bone strength. They were thought to be a disease of the elderly, but it is now increasingly recognized at younger ages due to the longer survival of chronically ill children and exposure to skeletal toxic treatments.[10]

In the regulation of osteoblasts and osteoclasts, there are plenty of proinflammatory cytokines. FMF, an unusual chronic inflammatory disease and inflammation in FMF patients, is thought to be related by cytokines.[11] Therefore, the aim of this study was to assess BMD in Egyptian children with FMF on genetic basis.

Patients and Methods

This cross-sectional study included 45 patients with familial Mediterranean fever (FMF) and 25 control Egyptian children of both sexes with an age range between 3-16 years old. Among the 45 patients, 21 (46.6%) were boys and 24 (53.3%) were girls. The patients were reclassified into two groups. Group I(A) consisted of 23 cases of FMF patients (10 boys and 13 girls), which were newly diagnosed and used colchicine for 1 month or less (to examine the effect of FMF and avoid the colchicine effect). Group I(B) consisted of 22 cases of FMF patients (11 boys and 11girls) that used colchicine for more than 6 months (to examine the effect of colchicine). Patients should be in-between their attacks (in remission). MEFV mutations were defined in patients using molecular genetics technique. The exclusion criteria were being on medications known to affect growth, maturation, bone mineral accrual such as steroids, anticonvulsant medications, heparin and methotrexate (in oncology doses), a history of long bone fractures (two or more fractures if aged 10-year or less, three or more fractures if aged >10 years), suffering abnormality of the skeleton or spine such as scoliosis, kyphosis, skeletal dysplasia by history, suffering from systemic lupus, inflammatory bowel disease, juvenile rheumatoid arthritis, or hepatosplenomegaly. The 25 cases in the control group (group II: 17 girls and 8 boys) were selected to reflect healthy, normally developing children with the same age range (3-16 years) and ethnicity of the patient groups. The control group should be distanced from the patient's first-degree relative and clear from MEFV gene mutations. The patients and control groups should be free of current or previous medical conditions known to affect growth, maturation, physical activity, or nutritional status. The study was conducted at the pediatric rheumatology clinic of Abo El-Rish Children Hospital (Cairo University) during September 2010 to March 2011.

The parents and the children from both groups (patients and control) were informed about the purpose of the study and written consent was obtained. The study protocol was approved by the Ethics Committee of the National Research Centre.

The clinical data were registered on a standardized form that included age, gender, familial consanguinity, and history of the disease with special emphasis on colchicine treatment (the dosage and age of initiation and its response).

Mutation Analysis

To confirm the diagnosis, molecular examination of the patients and control groups were performed in the laboratory of the medical genetics department at Cairo University. Fresh blood (2 ml) was collected and placed in EDTA tube. The FMF gene mutations were tested using the FMF Strip Assay™ (ViennaLab, Labordiagnostika GmbH, Vienna, Austria). The assay covers 12 most recurrent MEFV gene mutations simultaneously: E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S and R761H.

BMD Measurements

Bone mineral density was measured in all patients and controls by dual energy X-ray absorptiometry (DEXA, Norland XR-46) at both the proximal femur and lumbar spines (L_2-L_4). The instruments were calibrated daily according to the manufacturer's instructions. Weight, height, age, and sex of each patient were used to estimate BMD (expressed as gm/cm^2 and standard deviation scores) and compared with the BMD values of controls. BMD were expressed as Z-score, which represents the value for standard deviation above or below the age and sex matched mean reference value. According to the BMD Z-scores at the femur and lumbar spines, patients were subdivided into normal, at risk of osteopenia (borderline), osteopenia, at risk of osteoporosis (borderline) and osteoporosis.[12]

Statistical Analysis

Collected data were complied, coded, verified and analysis was performed using the SPSS software version16. Frequency distribution of MEFV gene mutation was studied. Descriptive statistics (mean±standard deviation) were calculated for bone mineral density. Student's t-test used to compare between mean of two sets of numerical (parametric) data. ANOVA (analysis of variance) was used to compare between more than two sets of numerical (parametric) data. The Chi-square test was used to compare between qualitative data of two sets or more. Standards of probability were set to P<0.01 (considered highly significant) and P<0.05 (considered statistically significant) for all analyses.

Results

The frequency distribution of FMF gene mutations among patients and controls is shown in Table 1. The most frequent gene mutation in

the patient group was E148Q heterozygous (35.6%), V726A heterozygous (33.3%), and the least common was M694V homozygous which was detected in 1 case only (2.2%). In spite of the absence of mutations in 15 control children (60%), there were 4 heterozygous gene mutations in 10 control children (40.0%). The most frequent gene mutation in the control group was V726A (16.0%), followed by E148Q (12.0%), M680I (8.0%), and M694V (4.0%).

Comparison between BMD and corresponding Z-score of the three study groups is presented in Table 2. There were significant differences in BMD at the femur and lumbar spine between the three groups (P_4<0.05). Control children had normal values of BMD at the femur and lumbar spines. Patients receiving colchicine treatment for less than 1 month (pharmaceutical effect of colchicine appears after its regular use for at least 6 months) had highly significant lower values of BMD at the femur and lumbar spines than the control children (P_2<0.05). Patients receiving colchicine treatment for more than 6 months had improved values of BMD at the femur, as there were insignificant differences between them and the control group. However, there were still significant differences between them at the lumbar spine (P_1>0.05). In both groups of patients, there were significant improvements in BMD at the femur among patients receiving colchicine treatment for more than 6 months. However, the improvement at the lumbar spines were still insignificant than patients receiving colchicine treatment for less than 1 month but still at lower values. This means that FMF disease had a significant effect on BMD at the femur and lumbar spine, however, regular use of colchicine treatment improves this effect (mainly at the femur) almost up to normal values.

Nobody in our sample (patients and control) had osteoporosis. However, comparisons between the frequency of the risks of osteopenia and osteopenia at the femur and

Table 1: Frequency distribution of FMF gene mutations in patients and controls

Gene	(N (%))	
	FMF patients (n=45)	Controls (N=25)
E148Q	16 (35.6) heterozygous	3 (12.0) heterozygous
V726A	15 (33.3) heterozygous	4 (16.0) heterozygous
M680I	13 (28.9) heterozygous	2 (8.0) heterozygous
M694V	1 (2.2) homozygous	1 (4.0) heterozygous
No mutation	0 (0.0)	15 (60.0)

Table 2: Comparison between BMD and corresponding Z-score of the three study groups

Bone mineral density	Mean±SD			P_1	P_2	P_3	P_4
	Patients (N=45)		Control (N=25)				
	Colchicine>6 months (N=22)	Colchicine<1 month (N=23)					
BMD femur neck	0.74±0.13	0.62±0.12	0.74±0.16	0.982	0.002	0.004	0.003
BMD Troch.	0.60±0.11	0.53±0.11	0.64±0.19	0.397	0.007	0.068	0.023
BMD Ward's triangle	0.64±0.135	0.54±0.12	0.63±0.15	0.756	0.020	0.011	0.020
BMD lumbar spine 2	0.57±0.13	0.51±0.10	0.68±0.21	0.002	0.000	0.239	0.001
BMD lumbar spine 3	0.59±0.12	0.52±0.11	0.68±0.21	0.036	0.000	0.150	0.002
BMD lumbar spine 4	0.56±0.11	0.50±0.10	0.66±0.20	0.020	0.001	0.241	0.002
BMD lumbar 2-4	0.57±0.12	0.51±0.10	0.67±0.20	0.025	0.000	0.196	0.002
Z-score BMD femur neck	−0.08±0.59	−0.47±0.48	0.01±0.59	0.572	0.004	0.022	0.010
Z-score BMD lumbar spine	−0.13±0.68	−0.08±0.77	0.25±0.59	0.062	0.095	0.824	0.119

P_1: Significance between the control group and patients who received colchicine for more than 6 months, P_2: Significance between the control group and patients who received colchicine less than 1 month, P_3: Significance between a group of patients who received colchicine for more than 6 months and those receiving it for less than 1 month, P_4: Significance between the control group and both patient groups (P<0.05 significant, P<0.01 highly significant)

Table 3: Comparisons between the risks of osteopenia in the three groups

BMD grade	N (%)			P_1	P_2	P_3	P_4
	Colchicine>6 months (N=22)	Colchicine<1 month (N=23)	Control (N=25)				
BMD grade femur							
Normal	16 (72.72)	14 (60.68)	24 (96)	0.046	0.007	0.482	0.025
Risk of osteopenia	4 (18.8)	7 (30.43)	1 (4)				
Osteopenia	2 (9.09)	2 (8.69)	0				
BMD grade spine							
Normal	17 (77.27)	16 (69.65)	25 (100)	0.028	0.017	0.868	0.009
Risk of osteopenia	2 (9.09)	5 (21.73)	0				
Osteopenia	3 (13.63)	2 (8.69)	0				

P_1: Significance between the control group and patients who received colchicine for more than 6 months, P_2: Significance between the control group and patients who received colchicine less than 1 month, P_3: Significance between a group of patients who received colchicine for more than 6 months and those receiving it for less than 1 month, P_4: Significance between the control group and both patient groups (P<0.05 significant P<0.01 highly significant)

lumbar spine revealed a significant difference between the three groups. The control group showed significant differences in frequency of osteopenia at the femur and lumbar spine compared with both groups of patients. However, insignificant differences were detected between the two patient groups. The risk of osteopenia in the neck femur (30.3%) and the lumbar spine (21.7%) was significantly more frequent in the patient group receiving colchicine less than 1 month, due to the effect of FMF itself (Table 3).

Comparisons between the effects of types of gene mutations on the BMD of 45 patients revealed insignificant differences in BMD at the femur and lumbar spines (Table 4). The effect of colchicine also had no relation with the type of genes mutation. Moreover, different gene mutations among the patients showed insignificant differences in the risk of osteopenia (Table 5).

Discussion

Children with inflammatory diseases are at the risk of low bone mass because of the complex effects of cytokines, glucocorticoid therapy, malnutrition, delayed pubertal maturation, low muscle mass and physical inactivity. These factors converge to influence bone homeostasis and create a musculoskeletal phenotype characterized by low trabecular BMD and weak cortical bone.[13]

The present study revealed predominance of four rather than a single gene mutation among Egyptian children. The most frequent gene mutation in the patient group was E148Q heterozygous (35.6%), V726A heterozygous (33.3%), M680I heterozygous (28.9%) and the least common was M694V homozygous, which was detected only in one case (2.2%). This coincides with the previous studies,[2,14] which reported the diversity of mutations among Arabs. Talaat et al.,[2] reported that E148Q,

Table 4: Comparisons between the effects of gene mutation types on the BMD of 45 patients

Bone mineral density	Mean±SD			F	P
	E148Q (N=16)	V726A (N=15)	M680I (N=13)		
BMD femur neck	0.66±0.14	0.68±0.12	0.68±0.16	0.367	0.777
BMD femur trochanter	0.55±0.10	0.56±0.11	0.57±0.14	0.584	0.629
BMD wards triangle	0.57±0.12	0.58±0.12	0.60±0.17	0.250	0.861
BMD lumbar spine 2	0.52±0.14	0.56±0.08	0.53±0.11	0.848	0.476
BMD lumbar spine 3	0.51±0.13	0.58±0.10	0.55±0.12	1.39	0.257
BMD lumbar spine 4	0.51±0.11	0.55±0.10	0.53±0.13	0.746	0.531
BMD lumbar 2-4	0.51±0.13	0.65±0.09	0.54±0.12	1.00	0.401

P<0.05 significant, P<0.01 highly significant

Table 5: Comparisons between the risks of osteopenia in patients with different gene mutations

	N (%)			P
	E184Q heterozygous (N=16)	V726A heterozygous (N=15)	M680I heterozygous (N=13)	
BMD grade femur				
Normal	11 (68.8)	11 (73.2)	8 (61.5)	0.472
Risk of osteopenia	4 (25.0)	3 (20)	4 (30.8)	
Osteopenia	1 (6.3)	1 (6.7)	1 (7.7)	
BMD grade lumbar spine				
Normal	9 (56.3)	14 (93.3)	10 (76.9)	0.067
Risk of osteopenia	4 (25)	1 (6.7)	2 (15.4)	
Osteopenia	3 (18.8)	0	1 (7.7)	

V726A, and M680I mutations were the most common mutations seen in the heterozygous group and were found in 11/40 patients (27.5%), 8/40 patients (20%), and 6/40 patients (15%), respectively. El-Garf et al.,[15] also reported that the most frequent gene mutation among a group of Egyptian patients suffering from FMF was V726A in 41.2%, followed by M694V, M680I and E148Q in 32.4%, 29.4% and 25%, respectively. This could be related to the heterogeneous origin of the Egyptian population and the marked effect of different civilizations (such as Romans, Byzantines, and Ottomans beside the original Arab inhabitants) on Egypt since the ancient times; exacerbated by its unique location at the crossroads between Africa, Europe, and Asia.[16]

However, the most frequent mutations among the Arabs of North Africa were M694V and M694I.[17] In Syrian patients, M694V and V726A mutations were the most common mutations in 45.8% and 26%, respectively.[18] In Turkey, Ceylan et al.[4] also reported that a single predominant mutation was not found in FMF patients among the Turkish population. They found that M694V was the most frequent mutation (28%), followed by V726A (11.7%), E148Q (9.1%), and M680I (G/C) (3.9%) in the Central Anatolian population. Furthermore, Ozturk et al.[16] and Touitou[19] reported the same order of FMF gene mutation among Turkish.

In this study, in spite of the absence of mutations in 15 control children (60%), there were four heterozygous gene mutations in 10 control children (40.0%). The most frequent gene mutation in the control group was V726A (16.0%), followed by E148Q (12.0%), M680I (8.0%) and M694V (4.0%). These findings were not reported before in other studies. By definition, anyone with two copies of MEFV mutations is considered to have FMF, but the situation is more complex for individuals with one MEFV mutation. Heterozygous patients should have more strong clinical evidence regarding FMF because they can actually have FMF or merely be simple carriers.[18] Therefore, molecular genetic analysis could be used for early and correct diagnosis of FMF in children with suspected manifestations. It helps to make decisions for the initiation of lifelong prophylactic treatment with colchicine, which is a very effective drug in treating FMF and preventing febrile attacks and amyloidosis in both children and adults.[20,21]

In this study, comparisons between BMD and the risk of osteopenia in the neck femur and lumbar spine, revealed significant differences between the three groups. The control group showed significant differences in BMD and frequency of osteopenia at the femur and lumbar spine with both groups of patients. This is in agreement with a study by Yildirim et al.,[11] who demonstrated a reduction of BMD in the lumbar spine, femoral neck, and total femur in adult FMF patients. The study by Duzova et al.,[22]

they showed that FMF patients had significantly lower BMD values than the control group. They also reported that the lumber measurements exhibited significant reduction in BMD than femoral values when average BMD scores were compared. It is known that trabecular bone loss is more prominent than cortical bone loss in osteopenia and osteoporosis. Therefore, it is expected that BMD might be lower in the lumbar region, which is rich in trabecular bone when compared with the femoral area.[11]

In the present study, patients who received colchicine treatment for more than 6 months had improved values of BMD at the femur as there were insignificant differences between them and the control group (P=0.98), but there were still significant differences between them at the lumbar spine (P=0.025). However, insignificant differences were detected between both groups of patients. The risk of osteopenia in the neck femur (30.3%, P=0.007) and lumbar spine (21.7%, P=0.017) was significantly more frequent with patient group receiving colchicine less than 1 month. Therefore, the above-mentioned difference regarding Z-scores was caused by the FMF inflammatory activity and subclinical inflammation. This is in agreement with Suyani et al.,[23] who reported that regular colchicine treatment, which might have suppressed the inflammatory status of FMF, lead to improved BMD values. In contrast, Berkdemir et al.[24] reported insignificant difference between the patient and control groups regarding median values of the lumbar and femoral BMD and their Z-scores.

The chronic inflammation associated with FMF has been shown to be an important risk factor in the development of decreased BMD. It has been suggested that local or systemic inflammatory cytokines release in arthritic joint might be involved in bone loss,[11,13,24] and influence linear growth of children by their systemic effects and local effects on growth cartilage of long bones.[13] A support for this hypothesis has come from some studies,[22,23] which demonstrated decreased BMD values in the spine of children with FMF.

Conclusion

FMF disease had a significant effect on BMD at the femur and lumbar spine, while regular use of colchicine treatment improves this effect (mainly at the femur) almost up to the normal values. However, there is insignificant effect of gene mutation type on BMD and the risk of osteopenia among the patients. There is predominance of four gene mutations among Egyptian children,

namely E148Q, V726A, M680I, and M694V. There were four heterozygous gene mutations in 40% of the control children, however, this requires more advances studies regarding the high prevalence of carrier rate. Molecular genetic analysis could be used for an early and correct diagnosis of FMF in children with suspected manifestations. It is recommended that regular intake of colchicine would improve bone health in FMF patients.

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