Clinical and molecular evaluation of children with Familial Mediterranean Fever and their siblings: A single center study in Upper Egypt.

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Abstract

Background: Familial Mediterranean Fever (FMF) represents the most prevalent monogenic auto inflammatory disease, with its etiology primarily attributed to mutations within the MEFV gene.

Objective: To characterize the clinical patterns of FMF, identify common gene mutations, and monitor the response to therapy in children suffering from FMF and their siblings.

Patients and Methods: The Pediatric Department of Sohag University Hospital served as the setting for this one-year prospective observational study (August 2022 - August 2023). Children and their siblings, all diagnosed with Familial Mediterranean Fever (FMF) based on Tel Hashomer criteria, were included in the research.

Results: A total of 99 children were included (85 children already diagnosed and 14 of their siblings). Patients were divided into 3 groups: negative- gene group(n=16), homozygous group(n=30) and heterozygous group(n=53). Fever and abdominal pain were the most common presentations in 96.9% and 93.9% of patients, respectively**.** Homozygous group had significantly higher severity score than other groups (p value=0.048). Response to colchicine therapy was complete in 82.8% of patients, incomplete in 16.1% and no response in one patient. The most frequent mutations were M-694-V, M-694I, E-148-Q and V-726-A reported in 30.12%,25.30%,15.66% and 10.84%. of patients, respectively.

Conclusion: In our cohort study, fever and abdominal pain were the most common presentations of FMF and the most frequent mutations were M-694-V, M-694I, E-148-Q and V-726-A. Most of newly diagnosed patients had the same genetic mutation as their siblings. Patients in homozygous group had higher severity score than other patients. Most patients achieved complete response to therapy with favourable response noted with E148Q mutations followed by M694V mutations.

Keywords: genetic mutations, familial Mediterranean fever, colchicine.

Introduction

The most common inherited autoinflammatory condition, Familial Mediterranean Fever (FMF), is triggered by mutations in the MEFV gene on chromosome 16. Its high prevalence is particularly evident in those of Mediterranean and Middle Eastern heritage, where incidence rates fluctuate between 1/200 and 1/1000. Strikingly, the carrier rate among Armenians reaches approximately 20%, meaning one in five people carry the gene. (1)

Clinically, FMF presents with recurrent, self-resolving attacks of fever and serositis, often starting during childhood. The underlying genetic cause is mutations in the MEFV gene, which codes for pyrin, a protein essential for pyrin inflammasome formation. This inflammasome, when activated by pathogens or cellular damage, triggers the release of IL-1β and IL-18. In FMF, mutated pyrin leads to aberrant, antigen-independent inflammation. While initially considered an autosomal recessive disorder, FMF exhibits significant variability. It can occur in individuals with a single MEFV variant or even without detectable variants. Furthermore, some families demonstrate a pattern resembling dominant inheritance. (2)

Pathogenic mutations in the MEFV gene, which contains 10 coding exons, are most frequently found in exon 10. In contrast, variants located in exon 2 are usually benign or classified as variants of uncertain significance (VUS/VOUS), and when present, tend to result in a less severe form of the disease. (3)

Several diagnostic tools, in the form of clinical criteria, have been created to support the diagnosis of FMF and to differentiate it from other autoinflammatory conditions. These include Eurofever, Tel-Hashomer, simplified Livneh, Turkish pediatric criteria, and Eurofever/PRINTO. However, it's important to note that a single, globally recognized diagnostic standard is not yet established. (4) The 2015 SHARE recommendations for pediatric rheumatology emphasize that genetic testing serves as a supportive tool in FMF diagnosis, complementing clinical evaluation. A negative genetic test does not preclude a diagnosis of FMF. Consequently, the clinical phenotype remains the primary determinant in FMF diagnosis. However, clinicians must be aware of the significant variability observed in genotype-phenotype correlations within FMF patients. (5)

The prevalence of clinically diagnosed FMF in patients with one or no MEFV gene variants has driven research towards understanding the role of other factors in disease expression. Current hypotheses include the involvement of modifier genes leading to oligogenic inheritance, the influence of still-unknown environmental triggers on MEFV variant penetrance, and the impact of epigenetic mechanisms, particularly gene methylation, on inflammasome gene activity and disease severity(6).

For individuals with FMF, colchicine is the primary therapeutic intervention. It functions by decreasing the frequency, duration, and intensity of FMF attacks, and by suppressing ongoing subclinical inflammation, thereby preventing the development of amyloidosis(7). However, roughly 5% of FMF patients demonstrate resistance to colchicine, characterized by one or more monthly attacks

despite six months of maximum tolerated dosage. In these colchicine-resistant cases, consideration should be given to the use of anti-IL-1 therapies. [8]

The purpose of this study was to characterize the clinical manifestations of FMF, to identify common MEFV gene variants, and to monitor the response to treatment in pediatric patients and their siblings diagnosed with FMF at Sohag University Hospital.

Ethical consideration:

The study protocol was approved by the Medical Research Ethics Committee -Faculty of Medicine- Sohag University (Registration number: Soh-Med-22-07-20).

Informed written consent was obtained from the parents/care giver of the children to participate in the study.

Confidentiality of the patient data and results of the study were preserved

The patient has the right to refuse or withdraw from the study at any time

No conflict of interest regarding the study or publication

Authors declared that there is no fund regarding the study or publication

Sample size Equation:

Based on previous research [9] ,the prevalence of FMF in Arab children was 1 per 2600 ,the sample size was calculated according to the following equation:

$N= Z^2\ p(1-p)/d^2$ (Daniel, 1999) [10]

N=desired sample size,

Z=the statistic corresponding to the level of confidence (1.96)

P=expected prevalence (0. 038)

d =precision (d is considered 0.05 to produce good precision and smaller error of estimate)

The sample size by the equation was at least 56 patients and we increased to 99 patients to overcome dropout

Inclusion Criteria:

All children diagnosed with FMF according to Tel Hashomer criteria and aged below 18 years.

Both males and females

Siblings of already diagnosed FMF patients

Exclusion Criteria:

Children suffering from other auto-inflammatory conditions

Healthy carriers of FMF genes

Children suffering from various illnesses

Study procedure:

This was a prospective observational study conducted over one year (August 2022 to August 2023) at the Pediatric Department, Sohag University Hospital. It included 99 patients with FMF and their siblings.

The study included all children diagnosed with FMF according to Tel Hashomer criteria and aged below 18 years. In addition, we screened siblings of already diagnosed FMF patients for FMF by clinical evaluation, laboratory and genetic testing. Children already diagnosed with FMF and their siblings who diagnosed later on were included in the study Healthy carriers of FMF genes were excluded from the study also children suffering from various illnesses or other auto-inflammatory conditions were excluded.

The Tel-Hashomer criteria [11] help doctors to diagnose FMF using "major" and "minor" symptoms. Major symptoms include; recurring fevers with serositis, amyloidosis without predisposing cause, and improvement with colchicine. Minor symptoms include; recurring fevers, skin rash that looks like erysipelas and a family history of FMF. If a patient has two major symptoms, or one major and two minor symptoms, they are diagnosed with FMF. If they have one major and one minor symptom, they are considered to have probable FMF.

Participants underwent a detailed assessment including the followings:

I-Clinical history including socio-demographic data (age, sex, residence, consanguinity), family history of FMF, duration of the disease, onset, course, and frequency of attacks per year, dose of colchicine and effect of treatment.

II-Meticulous clinical Examination was done for all participants with focus on abdominal examination.

II- Severity of FMF was assessed according to Tel Hashomer Severity Score [12] including the following:

1-Age at onset in years(<6=4points,6-10=3 points,11-20=2points)

2-Number of attacks per months(< 1=1 point,1-2=2 points, > 2=3 points)

3-Presence of arthritis(acute=2 points, protracted=3 points)

4-Presence of erysipelas like erythema(2 points)

5-Presence of amyloidosis(3 points)

6-colchicine dose in mg /day(1 mg =1 points, 1.5 mg=2 points, 2mg=3 points,> 2mg=4points)

According to this score disease is classified into mild disease (2-5 points); moderate disease (6-10 points); severe disease (>10 points).

III- Laboratory evaluation including:

Complete blood picture (CBC) was measured by automated blood cell counter analyzer Sysmex XN 1000 (Sysmex Corporation, Kobe, Japan).

CRP (C-Reactive Protein) on Cobas c311 Chemistry Analyzer System (Roche Diagnostic, GmbH, Mannheim, Germany).

ESR (Erythrocyte Sedimentation Rate) using the Westergren method.

Amyloid A Level measured by FinecareTM FIA Meters is a fluorescence immunoassay for quantitative measurement (SAA) (Guangzhou Wondfo Biotech Co, Guangzhou, P.R. China)

FMF Gene Analysis: A sample of DNA was obtained from a blood sample. The procedure included single multiplex PCR for the amplification of relevant sequences in the relevant FMF gene sequences followed by reverse hybridization of biotinylated amplification products to oligonucleotides probes on the test strip. The assay covered the following 12 MEFV mutations: E148Q ,M694V ,M694I,V726A, M680I(G/C), M680I(G/A),P369S ,F479L ,I692del ,K695R, A744S,R761H.

According to gene analysis our patients were classified into three groups

-Group (1) : patients with negative negative-MEFV gene mutations

-Group (2) : patients with homozygous genotype

-Group(3): patients with heterozygous genotype

Statistical analysis

We conducted all statistical analyses using IBM SPSS Statistics (version 22.0). To determine the distribution of continuous variables, we used the Kolmogorov-Smirnov test. Normally distributed continuous data are reported as the mean and standard deviation (mean ± SD), while non-normally distributed data are reported as the median and interquartile range (median (IQR)). Categorical variables are summarized as frequencies and percentages. We compared groups using independent t-tests for normally distributed continuous data, Mann-Whitney U tests for non-normally distributed continuous data, and Chi-square tests for categorical data. A p-value less than 0.05 was considered statistically significant.

Results

The study included 99 children (57 males and 42 females) with mean age of 7.41 ± 3.41 years and mean age at diagnosis of 5.82 ± 2.64 years. Of them, 85 children already diagnosed with FMF and 14 of their siblings who diagnosed later on with FMF. Twenty healthy carriers (according to clinical data) of siblings of FMF patients were excluded from the study. In addition, no subclinical cases (individuals with raised inflammatory markers but no clinical symptoms) were identified. The newly diagnosed FMF patients were siblings of 11 from already diagnosed patients.

Table (1): Baseline demographic Characteristic of the studied groups.

Parameter	Group**1 (n = 16)gene negative**	Group**2(n = 30) Homozygous**	Group**3(n = 53) Heterozygous**	**P-value**
Age /Years\Mean + SD	3.94 ± 1.6	8.27 ± 1.8	7.98 ± 1.4	**< 0.001***
P-value**	**1 vs 2 < 0.001**	2 vs 3 = 0.680	**1 vs 3 < 0.001**	
Sex				
Female	9 (56.2%)	9 (30%)	24 (45.3%)	0.190***
Male	7 (43.8%)	21 (70%)	29 (54.7%)	
FMF Family History	2 (12.5%)	16 (53.3%)	18 (34%)	**0.020*** **
Consanguinity	4 (25%)	17 (56.7%)	21 (39.6%)	**0.042*** **

*ANOVA test was used to compare difference in the mean between groups.; ** Post-hoc test with Bonferroni correction for pairwise comparisons.; *** Chi square test was used to compare the difference in frequencies among groups.; P value < 0.05 statistically significant.

According to MEFV gene mutations, patients were divided into 3 groups: group (1) negative-gene group which included 16/99 patients (7 males & 9 females), group (2) homozygous group which included 30/99 patients (21males & 9 females) and group (3) heterozygous group which included 53/99 patients (29males & 24 females). The mean age of patients in negative- gene group was statistically significantly lower than that of homozygous and heterozygous group (p value < 0.001). Positive parental consanguinity and family history of FMF were significantly higher in homozygous group than other groups. (**Table 1**).

Table (2): Frequency of Symptoms and Signs among the studied groups:

Symptoms(n(%)	Group**1 (n = 16) gene negative**	Group**2(n = 30) Homozygous**	Group**3(n = 53) Heterozygous**	P value
Fever	16 (100%)	30 (100%)	50(94.33%)	0.223
Abdominal Pain	13 (81.3%)	29 (96.7%)	51 (96.2%)	0.199
Chest Pain	4 (25%)	9 (30%)	10 (18.9%)	0.199
Arthritis	10 (62.5%)	20 (66.7%)	36 (67.9%)	0.199

Fever and abdominal pain were the most frequently reported symptoms, occurring in 96/99(96.9%) and 93/99(93.9%) of patients, respectively. Fever was documented in all patients of homozygous and negative gene group and in 94.3% of cases in heterozygous group. Abdominal pain was more frequent in heterozygous and homozygous groups than in negative gene group. Arthritis was reported in two-thirds of the patients, while chest pain was documented in 23.2% of cases. Arthritis was almost at the same percentage in all groups while chest pain was more frequent in heterozygous compared to other groups (**Table 2**).

Table 3 Diseases severity score, colchicine dose and response in the study participants.

Variable		Group**1 (n = 16) gene negative**	Group**2(n =30) Homozygous**	Group**3(n =53) Heterozygous**	P-value
Severity score		8.19±1.4	9.23±1.4	8.43 ±1.7	0.04*
Severity Degree	Mild	0	0	2 (3.7%)	0.35*
	Moderate	12 (75%)	17 (56.7%)	35 (66.03%)	
	Severe	4 (25%)	13 (43.3%)	16 (30.2%)	
Colchicine dose to control attacks mg/day(mean±SD)		0.56±.17	0.87±.32	0.74±.33	0.00**
Response to Therapy	complete	13(81.2%)	25(83.3%)	44(83.02%)	0.85*
	incomplete	3(18.7%)	5(16.7%)	8(15.09%)	
	No response	0(%)	0(%)	1(1.89%)	

Test used * chi square; ** student t test if parametric data. Mann Whitney U test if nonparametric data.
P-value≤0.05 is significant.

As regard to disease severity score, it was significantly higher in homozygous group than in heterozygous and negative gene groups (p-value=0.048). Mild disease was reported in 2/99(2.3%) and moderate in 46/99(46.4%) and severe in 33/99(33.3%). Severe disease was more frequent in homozygous group than heterozygous and negative gene groups (in 43.3%,30.2% and 25% of cases respectively). Patients required an average colchicine dose of 0.75±0.32 mg/day to control FMF attacks. Notably, the homozygous group required a significantly greater mean dose (0.87±.32 mg/day) than the other groups studied (p=0.008).Regarding response to colchicine therapy in our patients it was complete in 82/99 (82.8%), incomplete in 16/99 (16.1%) and no response in one patient (1.01%) (**Table 3**).

Table 4 -Mutations and alleles in study patients.

		Genotype (mutation)	N (%)
Homozygous(n=30,36.14%)		M-694-V	16 (19.27%)
		M-694I	11(13.25%)
		V-726-A	3(3.61%)
heterozygous(n=53)			
		E-148-Q	13(15.66%)
		M-694I	10(12.04%)
		M-694-V	9(10.84%)
		V-726-A	6(7.22%)
		P-369-S	1(1.20%)
		E-148-Q- V-726-A	3(3.61%)
		E-148-Q-M680I(G/A)	1(1.20%)
		E-148-Q- M-694I	3(3.61%)
		M694V and M694I	2(2.4%)
		M6801(G/C)-M680I(G/A)	1(1.20%)
		M-694I- V-726-A	2(2.4%)
		M-694I- M6801(G/A)	2(2.4%)

Analysis of MEFV gene mutations in our patient population showed that mutations were detected in 83 patients with the most common mutations were: M-694-V, found in 25 out of 83 patients (30.12%); M-694-I, found in 21 out of 83 patients (25.30%); E-148-Q, found in 13 out of 83 patients (15.66%); and V-726-A, found in 9 out of 83 patients (10.84%). In the homozygous group, the most frequent mutation was M-694-V detected in 16/30(53.3%) patients whereas E-148-Q was the most frequent mutation reported in the heterozygous group(13/53,24.5%) (**Table 4**).

Table (5)Comparison between FMF patients & their sibling regarding severity score and most common mutations

	Already Diagnosed FMF Patients(n=85)	Newly Diagnosed Siblings(n=14)	P value
Severity score	8.6±1,6	8.5±1.7	0.57
Gene mutation			
M694V	16	9	
M694I	18	3	
E148Q	12	1	
V726A	8	1	
others	31	0	0.004

In the current study, 14 newly diagnosed FMF patients were siblings of 11 patients among 85 patients who were already diagnosed with FMF. There was no significant difference between FMF patients who already diagnosed and their siblings who diagnosed later on regarding severity score. A total of 9/14 (64.3%) of newly diagnosed patients had the same genetic mutation as their siblings with the most common mutation among patients and their sibling was M694V.(Table 5)

Table 5: comparison between the most frequent 4 mutations.

Variable		M694V N=25	M694I N=21	E148Q N=13	V726A N=9
Response	Complete	21(84%	15(71.4%)	12 (92.3)	6 (66.7%)
	Incomplete	4(16%)	6(28.5%)	1 (7.7%)	3 (33.3%)
Severity score		8.77±1.48	9.17±1.30	8.46±2.11	8.22±1.64
Colchicine dose to control attacks in mg/day (mean±SD)		0.81±.33	0.83±.34	0.73±.33	0.89±.49
Severity	Mild	1(4.6%)	0	0	0
	Moderate	16(46%)	13(61.9)	8(61.5%)	6(66.7%)
	Severe	8(32%)	8(38,09%)	5(38.5%)	3(33.3%)

Table 5 shows comparison between the most frequent 4 mutations in our cohort regarding severity score, dose of colchicine and response to therapy. Analysis of colchicine response revealed that patients carrying the E148Q mutation exhibited the most favorable outcomes, with 92.3% achieving complete remission and requiring a lower mean colchicine dose of 0.73±.33 mg/day. Furthermore, a significant proportion (84%) of patients with the M694V mutation achieved complete response to therapy. In contrast, the M694I mutation was associated with increased disease severity, as evidenced by 38.09% of patients in this group experiencing severe disease.

Discussion

Given that FMF is the most common monogenic autoinflammatory disease, and its prevalence is notable among Egyptian patients[13], the paucity of data specific to Upper Egypt is a significant gap. This study addresses this gap by providing the first comprehensive analysis of clinical features, genetic mutations, and therapeutic responses in children with FMF and their siblings at Sohag University Hospital in Sohag, Upper Egypt.

The mean age at diagnosis of our patients was 5.82 ± 2.64 years. This was consistent with other studies[13,14], However, it was lower than others [15,16] , probably due to early diagnosis practices at our department. The gender distribution in this study showed a slight male predominance , consistent with earlier reports [17,18]. A slight female dominance was noted in other studies [13,14]. On the other hand, Sayarlıoğlu M et a.,l[19] study reported FMF in both sexes in a similar ratioas.

Family history of FMF emerged as a crucial factor in disease diagnosis. In the current study 36.3% of patients has a family history of FMF. This was in agreement with Tanatar et al[20] Additionally, parental consanguinity was reported in 42.3 % of our patients with consanguinity rates were significantly higher among homozygous and heterozygous patients than in negative gene group, potentially due to cultural practices and genetic predispositions, as noted in studies by Duşunsel et al [15] and Settin et al[18] .

Regarding the clinical presentation of FMF, fever and abdominal pain were the most common presentations in 96.9% and 93.9% of our patients, respectively, followed by arthritis in 66% of patients and chest pain in 23.2% of cases. These results closely resembled those reported by other studies [13,21,22,23]. Ebrahimi-Fakhari et al. [24] documented abdominal pain in 95%, fever in 78% of patients, , arthritis in 59%, and chest pain in 32%. A large Turkish cohort study revealed a distinct symptom profile, with abdominal pain (88.2%) slightly more common than fever (86.7%), and noted arthritis (27.7%), chest pain (20.2%), myalgia (23%), and erysipelas-like erythema (13.1%), which was not observed in our patient group. The variable presentation of FMF, as evidenced by the fluctuating prevalence of abdominal pain and fever in different studies, reflects the complex nature of this autoinflammatory disorder.

Our study revealed a distribution of FMF severity with 2.3% mild, 46.4% moderate, and 33.3% severe cases. While the severity distribution in our cohort was similar to that observed by Talaat et al., [13] who reported 10.53% mild, 64.21% moderate, and 25.26% severe cases, it differed substantially from Almaalky et al.'s[14] findings. They reported a higher prevalence of mild cases (52.7%) and a lower prevalence of moderate cases (10.9%), with severe cases at 36.4%.

The severity of FMF in our patient cohort was significantly influenced by MEFV genotype. Patients with homozygous variants demonstrated the highest severity scores (9.23±1.4), a finding that was statistically significant compared to heterozygous and gene-negative groups (p=0.048). This result aligns with Aktas et al.'s [25] observation that homozygous and compound heterozygous genotypes are associated with severe FMF. Furthermore, the observed gradient in

severity, with heterozygous patients exhibiting moderate severity (8.4) and gene-negative patients the lowest (8.1), is consistent with Duşunsel et al[15]. The overall pattern, where homozygous and heterozygous patients exhibited substantially higher severity than gene-negative patients, supports previous findings by Yilmaz et al. and Ozturk et al [26,27].

Colchicine remains the primary therapeutic intervention for FMF. In this cohort, 82.8% of patients achieved complete remission, 16.1% demonstrated partial response, and 1.9% showed no response to colchicine. These results are comparable to those by Duşunsel et al. [15]., who reported complete remission in 77.5% of patients. Notably, heterozygous patients required lower colchicine doses than homozygous patients, reflecting findings from Talaat et al. [13].

In the current study genetic analyses revealed that 16.1% of patients had no detectable mutations , 53.5% of patients had heterozygous mutations and 30.3% of patients had homozygous mutations, aligning with earlier findings from Mattit et al [28].

The most frequent mutations identified in this cohort were M-694-V, M-694I, E-148-Q and V-726-A, consistent with Tunca et al. [29] and Almalky et al study [14] but different from Linka et al., [30] study and El Gezery et al. [31] .The variation in mutation frequencies between this study and others could be attributable to differences in genetic sampling methods or regional genetic diversity.

In the current study, 14 newly diagnosed FMF patients were siblings of 11 patients among 85 patients who were already diagnosed with FMF. A total of 9/14 (64.3%) of newly diagnosed patients had the same genetic mutation as their siblings with the most common mutation among patients and their sibling was M694V . This came to an agreement with Arslanoglu Aydin et al., [32] in their study of 143 FMF patients and their sibling as they reported that 72% of the patients had the same genetic mutation as their siblings with the most common mutation was M694V present in 97/143(67.8%) of patients.

Our analysis of MEFV mutations revealed that patients with the E148Q mutation exhibited the most favorable response to colchicine therapy, with 92.3% achieving complete remission and requiring a low daily dose (0.73±.33 mg/day). This finding is consistent with observations from Talaal et al., [13] who also reported a superior response to colchicine in patients with the E148Q mutation. Furthermore, Aydin et al. [33] supported these findings, noting that the E148Q mutation is associated with a milder disease course and excellent colchicine response. Regarding the M694V mutation, 86.4% of patients achieved complete remission, which is comparable to Shrateh et al. [34] study, where 63% of patients reported improvement. Finally, 66% of patients with the V726A mutation demonstrated complete remission, which is in line with Shrateh et al. [34], findings of 50% improvement.

In the current study patients with M694I mutation had a higher severity score (9.17±1.3) than other patients and received higher doses of colchicine to control attacks of FMF. In Talal et al., study [13] only 14.29% % of patients with M694I achieved complete response. Contrary to our findings that most of patients with M694V mutation had mild to

moderate disease, El Beshlawy et al., study [35] showed that M694V mutation is associated with a severe phenotype and amyloidosis. This ensures that different genotypes affect the variability of the disease severity and response to treatment.

Limitations

Limitations include relatively small sample size, its single-center setting, a relatively short follow-up period and we screened for only 12 mutations.

Conclusion

Our study revealed that fever and abdominal pain were the primary clinical features of FMF in our cohort, with M-694-V, M-694I, E-148-Q, and V-726-A being the most common mutations. Siblings often shared the same genetic mutation. Homozygous patients exhibited significantly higher disease severity. Colchicine therapy was effective, particularly for patients with E148Q and M694V mutations.

Recommendation:

A larger, multicenter study with screening for more mutations and prolonged follow-up, is needed to validate the findings in this study.

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