Association Between Acute Phase Reactants (High Sensitivity C-Reactive Protein and Erythrocyte Sedimentation Rate) and the Genotype of Familial Mediterranean Fever (FMF) in Patients and Healthy Controls

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Research

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Abstract

Background: Familial Mediterranean fever (FMF) is an autosomal recessive disorder mainly common in Arabs, Non-Ashkenazi Jews, Armenians and Turks. The classical clinical features include painful attacks and recurrent acute fever with periods of remission.

Results: This study was carried out on 101 clinically diagnosed Syrian FMF patients, in addition to 107 apparently healthy controls. Twelve mutations in the gene locus of MEFV were detected using reverse hybridization and the M694V mutation was found to be the most common in Syrian patients. This study showed that there was a statistically significant difference between the two groups when comparing the levels of the high-sensitivity C-reactive protein (hs-CRP) and the erythrocyte sedimentation rate (ESR) for the two groups of patients and healthy controls. However, this study did not show a relationship between genotype of *MEFV* mutations and hs-CRP titers and ESR in these patients, as well as the absence of a strong relationship between the M694V mutation and hs-CRP titers and ESR.

Conclusion: These data indicate the importance of hs-CRP titers and ESR in patients with familial Mediterranean fever when diagnosing the disease, before starting treatment and in attack-free periods, which may give us an idea about the severity of the disease.

Background

Familial Mediterranean fever (FMF) (OMIM #249100) is an autosomal-recessive inherited disease. It is mainly common in Arabs, Jews, Armenians and Turks [1]. It is clinically characterized by recurrent episodes of inflammation and serositis which includes fever, peritonitis, pleuritis, synovitis or erysipelas-like erythema and may be complicated by AA amyloidosis [2].

The gene responsible for causing FMF is called *MEFV* that has been mapped to chromosome 16p13.3. It consists of 10 exons and encodes a protein called Pyrin which is expressed in granulocytes and negatively regulates inflammation. So far, about 379 variants have been associated with the *MEFV* gene [3]. Five founding mutations M694V, V726A, M680I, M694I, and E148Q are the most frequently encountered mutations in typical FMF patients [4]. Our previous study on 153 Syrian FMF patients showed that the M694V, V726A, E148Q, M680I (G/C) and M694I mutations account for 36.5, 15.2, 10.2, 13.2 and 10.2%, respectively [5]. The carrier rate in the Syrian population is 30.2% [6].

The clinical attacks are associated with high erythrocyte sedimentation rate (ESR) and acute-phase reactants as C-reactive protein (CRP), serum amyloid A (SAA), and fibrinogen. These entire laboratory parameters usually return to normal in the attack-free periods [7].

Acute phase reactants (APRs) (serum amyloid A protein (SAA), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR)) are used to monitor FMF [8]. It has been previously shown that the levels of CRP and SAA are correlated with each other during the FMF episodes. SAA has a significant importance in the identification of subclinical inflammation in FMF patients when other APRs were normal [9]. CRP

and SAA values were significantly higher among relatives of FMF patients (who were obligate FMF carriers) compared to the healthy controls [10]. A previous study showed significant increase in the levels of ESR and fibrinogen during attack-free periods in patients with the M694V mutation than in those without. However, CRP and leukocyte count did not show a statistical significant difference between those two groups [11].

The main aim of this study was to determine if there is an association between acute phase reactants (high sensitivity C-reactive protein (hs-CRP) and erythrocyte sedimentation rate (ESR)) and the genotype of familial Mediterranean fever gene (*MEFV*) in FMF patients at the time of diagnosis and before starting treatment with colchicine. Moreover, we tried to find if there is a link between M694V genotypes and the hs-CRP and ESR levels.

Results

A total of 208 blood samples were withdrawn from 101 FMF patients (group I) and 107 healthy controls (group II). The mean age was similar in groups I and II with no statistically significant difference (**23 ±** 11 in group I and **23.21 ±** 4.8 in group II) (Table 1). Values above the normal were detected for hs-CRP (> 6 mg/l) in 30 patients (30%) and for ESR (> 20 mm/1st h) in 29 patients (29%).

hs-CRP values ranged from 0.02 to 104 mg/L in group I and from 0.1 to 5.29 mg/L in group II. Mean hs-CRP was 7.97208 ± 18.58546 and 1.236075 ± 1.589378 mg/L in group I and II, respectively (Table 1). The *p*-value is 0.000243 which indicates a statistically significant difference between groups I and II.

ESR values ranged from 1 to 75 mm in 1 hour in group I and from 0.1 to 3.6 mm in 1 hour in group II. Mean ESR was 17.257 ± 19.403 and 1.317757 ± 1.213588 mm in 1 hour in group I and II, respectively (Table 1). The *p*-value is < 0.00001 which indicates a statistically significant difference between groups I and II.

Of the 101 FMF patients, mutations in the *MEFV* gene were identified in 65 (64.3%) patients and the mutation detection was negative in 36 (35.6%) patients. Of those 65 patients with mutations, 11 (17%) were homozygous, 28 (43%) were heterozygous, 19 (29%) were compound heterozygous and 7 (11%) were complex alleles. Mutation detection allowed the identification of 8 of different MEFV mutations out of the 12 tested mutations in 99 of the 130 tested alleles. The most dominant mutations detected were M694V (49%), V726A (17%), M694I (7%), E148Q (17%), M680I (G/C) (7%), R761H (1%), A744S (1%) and F479L (1%) (Table 2).

Of the total number of 101 FMF patients, 30 patients showed high hs-CRP. hs-CRP values ranged from 6.3 to 104 mg/L and mean hs-CRP was 23.73767 ± 28.3675. ESR values ranged from 3 to 72 and mean ESR was 25.83333 ± 19.59606. Of the 30 patients with high hs-CRP, MEFV mutations were identified in 21 (70%) and no mutation was identified in the remaining 9 (30%) patients. The most dominant mutations detected were M694V (47%), V726A (18%), M694I (11%), E148Q (11%), M680I (G/C) (11%) and R761H (2%) (Table 2).

Comparison of the MEFV genotype with the hs-CRP and ESR levels

In order to compare the *MEFV* genotype with the hs-CRP and ESR levels, 30 patients with high hs-CRP were divided into 4 groups: group A (no mutations), group B (heterozygotes), group C (compound heterozygotes) and group D (homozygotes +

complex alleles) (Table 3). We compared groups (A + B), (A + C), (A + D), (B + C), (B + D) and (C + D) using T-test for means. There were no statistical significant differences between the groups regarding the hs-CRP and ESR levels (data not shown).

Comparison of the different M694V genotypes with the hs-CRP and ESR levels

In order to compare the M694V genotypes with the hs-CRP and ESR levels**,** 19 patients with high hs-CRP were divided into 3 groups: group N (no mutations), group H (Compound heterozygote (M694V/Other) + Heterozygote (M694V/-)) and group Ho (homozygote (M694V/M694V)) (Table 4). We compared groups (N+H), (N+Ho) and (H+Ho) using T-test. There were no statistical significant differences between the groups when comparing the ESR and hs-CRP levels (data not shown), except ESR that showed a statistical significant difference between groups (H+Ho) (T-test 2.42).

Discussion

Different acute phase reactants (APRs) such as CRP, Serum Amyloid A (SAA) and ESR are used to monitor FMF patients [8]. CRP is readily accessible and affordable compared with SAA which is expensive and not widely available. Testing of SAA does not provide any additional information over CRP. Therefore, CRP seems to be adequate for follow-up of FMF patients [12]. CRP was the only APR increased in all attacks. Frequency of ESR was (88%), fibrinogen (63%), and WBC (50%) [13].

The aim of this study was to evaluate the association between acute phase reactants (hs-CRP) and ESR) and the genotype of familial Mediterranean fever gene (*MEFV*) mutations in FMF patients at the time of diagnosis and before starting treatment with colchicine. This may indicate if the hs-CRP and ESR can be used as cheap prognostic tools in evaluating FMF patients. To the best of our knowledge, this is the first report that discusses the importance of CRP in FMF patients upon diagnosis and before treatment.

Interestingly, this study showed a very strong association between hs-CRP and ESR and familial Mediterranean fever (FMF) compared with the healthy controls. This indicates the importance of evaluating hs-CRP and ESR in FMF patients when they are diagnosed and before starting the colchicine treatment and in the attack-free periods, which may give us an idea about the severity of the disease. A previous study showed that the white-blood cell count, CRP and IL-8 levels were higher in patients with FMF than in healthy subjects. Levels of ESR, fibrinogen, IgD, TNF-alpha, procalcitonin, IL-6, and C5a were not significantly different between patients and healthy subjects [14]. However, continuous elevation of acute phase reactants in FMF patients should be evaluated to prevent the development of amyloidosis [15].

In the current study, 12 known mutations in the MEFV gene were screened among 101 FMF Syrian patients which led to positive identification of 1, 2 or 3 mutations in 65 (64%) patients. The five most common mutations include M694V, V726A, M694I, E148Q, M680I (G/C) (Table 2). The five most common mutations detected in FMF patients with elevated hs-CRP include M694V, V726A, M694I, E148Q, M680I (G/C) (Table 2). The five most common MEFV mutations detected in this study are similar to what previously reported in the Syrian patients [5, 6, 16, 17] and the other Arab FMF patients [18] with different frequencies.

We Compared the *MEFV* genotype (group A (no mutations), group B (heterozygotes), group C (compound hetetrozygotes) and group D (homozygotes + complex alleles)) with the hs-CRP and ESR levels (Table 3). To the best of our knowledge, this is the first study that compares the *MEFV* genotype with the hs-CRP and ESR levels. We did not find any association between the *MEFV* genotype and the levels of hs-CRP and ESR. It has been previously shown that SAA and hs-CRP values were significantly increased in the patients with two mutations in exon 10 compared with the patients with one mutation in exon 10 which suggests an effect of gene dosage. Moreover, basal and peak concentrations of SAA and hs-CRP were highly increased in *MEFV* heterozygotes than in wild-type controls, regardless of mutation [7]. Kosan and colleagues have investigated the relationship between acute-phase reactants (ESR, CRP, fibrinogen, and white blood cell count) and gene mutations in attack-free periods of FMF children aged 2–18 years [11]. FMF patients' samples were collected every 6 months for two years. Mean values for erythrocyte sedimentation rate and fibrinogen were statistically significant difference in the homozygous group. White blood cell count and CRP were similar in both groups.

We compared the M694V genotypes (group N (no mutations), group H (Compound heterozygote (M694V/Other) + Heterozygote (M694V/-)) and group Ho (homozygote (M694V/M694V) with the hs-CRP and ESR levels (Table 4). There were no statistically significant differences between these groups when comparing the ESR and hs-CRP levels. A previous study reported the association between the different *MEFV* genotypes and clinical features and cytokine inflammatory activity during the attack-free period in FMF patients and compared with the healthy controls [14]. There were statistically significant differences in FMF patients than in healthy controls with regard to the levels of CRP and White-blood cell count (WBC). Moreover, there were no statistically significance difference between the patients with M694V homozygous and heterozygous genotypes in terms of the WBC, ESR, CRP, fibrinogen and procalcitonin. Another study showed that FMF children with M694V homozygote genotype were associated with a higher acute phase response during FMF attacks and severe clinical symptoms [19]. It should be noted that both studies reported the acute phase reactant levels either during the attack free period or the attack-periods and not at the time of diagnosis like what we did in this paper.

Limitations of this study are the small size of the groups and short-term follow-up. Although we used hs-CRP and ESR as they are widely available and not very expensive, other APRs should be used in any future research in order to compare the results with each other.

Conclusion

In conclusion, this study attempted to establish new aspects related to the spectrum of *MEFV* mutations in FMF patients and the levels of hs-CRP and ESR. M694V mutation was found to be the most common in Syrian patients. There was a strong association between *MEFV* gene mutations and the levels of hs-CRP and ESR. Levels of hs-CRP titers and ESR in FMF patients at the diagnosis of the disease, before starting treatment and in attack-free periods, may give us an idea about the severity of the disease. The study did not show a relationship between genotype of *MEFV* mutations and hs-CRP titers and ESR in these patients, as well as the absence of a strong relationship between the M694V mutation and hs-CRP titers and ESR.

Methods

This study was conducted in the Biochemistry and Microbiology Dept., Faculty of Pharmacy, Arab International University (AIU) between May 2019 and February 2020. A total of 101 unrelated FMF patients and 107 apparently healthy controls (53 males and 54 females) were included in this study. A clinical diagnosis of FMF was based on the Tel-Hashomer clinical criteria [20]. The inclusion/exclusion criteria for the FMF patients were as follows: 1) the FMF patients were diagnosed clinically, 2) the colchicine treatment has not been started yet, 3) there was no current FMF episode at the time of blood sampling and the last episode occurred in the more than two weeks ago.

For all FMF patients and apparently healthy controls, EDTA blood was sampled and DNA was isolated from frozen blood samples by the QIAprep Spin Miniprep Kit (Cat No./ID: 27104) according to the manufacturer's instructions (QIAGEN). *MEFV* mutation analysis was performed for 12 mutations using a reverse-hybridization assay (FMF StirpAssay™, REF 4-230) according to the manufacturer's instructions (ViennaLab Labordiagnostika, Vienna, Austria).

The Westergren method was used to determine the ESR (mm in 1 hour), and hs-CRP (mg/L) concertation was assessed by the turbidimetric method (Behring, Germany).

Statistical analysis

Values are expressed as the mean (± SD). Differences between groups were assessed using the *Z*-test for two proportions and T-test for means. A P value < 0.05 was accepted as statistically significant.

List Of Abbreviations

MEFV
Mediterranean fever gene.
Hs-CRP
High Sensitivity C-Reactive Protein.
ESR
Erythrocyte Sedimentation Rate.
FMF

Familial Mediterranean fever.
APRs
Acute phase reactants.
SAA
serum amyloid A protein.
WBC
White-blood cell count.

Declarations

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Authors' contributions

RAJ designed the study, analyzed the data and wrote the manuscript; WZ designed the study and revised the manuscript critically. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study has been approved by the Institutional Review Board of the Faculty of Pharmacy, Arab International University (AIU).

Every participant (patient and healthy control) was informed about the study and a written consent was signed either by the patient or his/her parent for blood sampling.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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Tables

Table 1
Comparison of means of age, ESR and hs-CRP

	FMF Patients	**Healthy Controls**	**T-Test**	***p*-value**	**Significant**
Total No.	**101**	**107**			
Mean of Age ± SD	**23 ±** 11	**23.21 ±** 4.8	**0.1802**	0.857173	No
Mean of ESR ± SD	17.257± 19.403	1.317757 ± 1.213588	8.4812	< 0.00001	yes
Mean of hs-CRP (mg/L) ± SD	7.97208± 18.58546	1.236075± 1.589378	**3.7351**	0.000243	yes

Table 2
The distribution of the MEFV mutations screened in 65 FMF patients and in 30 FMF patients with elevated hs-CRP

	MEFV Mutation	Number of mutations in 65 FMF patients (%)	Number of mutations in 30 FMF patients with high hs-CRP (%)
1	**M694V**	52 (**%49**)	18 (47%)
2	**V726A**	19)17%)	7 (18%)
3	**M694I**	8**7) %)**	4 (11%)
4	**E148Q**	18 (17%)	4 (11%)
5	**M680I (G/C)**	**7** (7%)	4 (11%)
6	**R761H**	**1** (1%)	1 (2%)
7	**A744S**	**1** (1%)	-
8	**F479L**	**1** (1%)	-
	Total	107 **(100%)**	38 (100%)

Due to technical limitations, table 3 is only available as a download in the Supplemental Files section.

Table 4 Comparison of subgroups according to the M694V mutation

	Group N	Group H	Group Ho
	No mutations	**(M694V/Other) Compound heterozygote + Heterozygote (M694V/-)**	**(M694V/M694V) Homozygote+ Complex alleles**
Number of patients	**9**	**5**	**5**
CRP (mean) ±SD	12.45 7.114861	10.8 8.112082	24.55 33.88965
ESR (mean) ±SD	26.22222 23.37615	53 22.12841	20.6 20.08233

Supplementary Files

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- Table3.jpg