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Research Article

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The Prevalence of Three Common MEFV Gene Mutations in West Bank Population among Students of Najah National University, Palestine

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Abstract

Background: Familial Mediterranean fever (FMF) is an autosomal recessive disorder. The clinical symptoms of FMF are nonspecific and difficult to distinguish from similar symptoms arising from completely different diseases. Following the cloning of the gene associated with this disease (*MEFV*), genetic analysis of its mutations has become available. Of these mutations, five account for more than 70% of FMF cases which are; V726A, M694V, M694I, M680I and E148Q. In this study, three out of the five common mutations of *MEFV* gene were analyzed in apparently healthy people in West Bank, Palestine.

Methods: We performed A cross sectional, non-interventional, descriptive study that aims to calculate the prevalence of three common *MEFV* gene mutations (M694V, M680I and V726A) in the West Bank population by taking a representative sample among An-Najah National University (NNU) students at the period between November 2013- January 2014. The research included a simple questionnaire and blood sampling searching for 3 common *MEFV* gene mutations.

DNA was extracted promptly using Phenol-Chloroform Isoamyl Alcohol (P-CIA) protocol. PCR methods were used to analyze the M694V, M680I and V726A mutations that have been previously defined by us to be three out of five commonest mutations worldwide.

Results: Overall, the prevalence of three common *MEFV* gene mutations in West Bank population among students of NNU was 23.5%. The most common mutation was V726A (12.7%), followed by M680I (8.3%), while the least one was M694V (2.4%).

Conclusions: In conclusion, the prevalence of the *MEFV* gene mutations was high and similar to neighbor countries such as Syria and Iran. According to these results, genetic counseling can play important role in reducing the number of affected patients, genetic screening in families with affected patients can reduce the risk of developing complications, as amyloidosis, by providing proper prophylactic with colchicine.

Keywords

Familial mediterranean fever; *MEFV* gene mutations; Genetic counseling

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Introduction

Familial Mediterranean Fever (FMF) is inherited inflammatory disorder, characterized by short, recurrent, apparently unprovoked attacks of fever and serositis, or erysipelas-like skin lesions [1]. FMF is the most frequent Periodic Febrile Syndrome among Auto-inflammatory Syndromes [2]. The disease is prevalent amongst populations surrounding the Mediterranean Sea such as Turks, Armenians, non- Ashkenazi Jews and Arabs [3].

The responsible gene (*MEFV*) was independently cloned by American and French groups in 1997 [4]. While the protein encoded by the *MEFV* gene has been named pyrin by an American group for its role in anti-pyrexia [5]. The *MEFV* gene is located on the short arm of chromosome 16 and includes 10 exons [4]. To date, More than 300 known mutations in the *MEFV* gene have been reported [6]. Of these mutations, five account for more than 70% of FMF cases which are; V726A, M694V, M694I, M680I and E148Q [7,8]. Forty-eight of the *MEFV* gene mutations are found in exon 10 [9]. Mutation E148Q in exon 2 was found to be the second most common mutation occurring in patients of several ethnicities with different haplotypes [9].

Symptoms of FMF are likely to start between the ages of five and fifteen, but could potentially start as early as infancy, or even appear later in life [10]. In fact, 90% of people experiencing FMF have been diagnosed before age twenty, and children under age ten make up 60% of FMF patients [11], the prevalence of FMF is rare in older than 40 years [11].

The FMF symptoms are divided into two major categories-common and rare manifestations [12]. The common manifestations included abdominal attacks, arthritis, arthralgia, pleural attacks, pre-attack symptoms, and amyloidosis; the rare manifestations were described as protracted febrile myalgia, erysipelas-like erythema, vasculitides, and chronic ascites [12].

However, the clinical symptoms of FMF are nonspecific and difficult to distinguish from similar symptoms arising from completely different diseases [13]. Following the cloning of the gene associated with this disease (*MEFV*), genetic analysis of its mutations has become available, providing a new tool for the establishment or confirmation of the diagnosis of FMF [4]. Furthermore, relatives with a high risk of FMF could be screened even before clinical manifestations and thus use a prophylactic treatment [14-17].

Only a screening study for *MEFV* gene mutations was performed on FMF patients within Palestinian population, 511 suspected patients were screened for 24 different *MEFV* gene mutations, the result revealed the presence of 14 different mutations from the screened 24 mutations; the study suggested that the origin of FMF among the Palestinian population is mostly homozygous [18]. However, the prevalence and distribution of *MEFV* gene mutations in apparently healthy people in West Bank population have not been studied. Therefore, we need a baseline study in Palestine to provide a database about *MEFV* gene mutations in apparently healthy people.

Aim

This study was initiated to investigate the prevalence of three

common *MEFV* gene mutations. i.e. M694V, M680I(G/C), V726A worldwide in healthy people of West Bank population, among students of NNU; in order to enriches the database about this mutations, as well as enhancing the genetic counseling processes.

Materials and Methods

In this cross-sectional, non- interventional, descriptive study, 450 students at NNU, who were attending the university clinics for reasons such as common colds or headaches were invited to participate during November 2013 - January 2014. This study was approved by the Ethics Committee of NNU and a signed consent was obtained from each participant. The study included apparently healthy students from west bank who were not diagnosed with FMF. For the sample to be representative of West Bank, the registration department in the University was conducted to find out the number of students from each governorate in West Bank. NNU which is located in Nablus; a large city in the north of Palestine educates 20,000 students in its campuses [19]. This large student's body is drawn from across Palestine, particularly West Bank governorates (Jerusalem, Ramallah, Hebron, Jericho, Nablus, Jenin, Tulkarem, Salfit, Tubas, Bethlehem and Qalqilya). Our estimated sample size was 255 students at most by using the following formula [20]:

$$n' = NZ2P(1-P) \setminus [d2(N-1)+Z2p(1-P)]$$

With n'=sample size with finite population correction, N=Population size (number of students at NNU at the first trimester in 2013 which was 20000) (35), Z=Z statistic for a level for confidence equals 95% (1.96), P=Expected proportion (0.181) [21] and d=Precision (0.05).

Taking risk factors in consideration through blood sampling, DNA extraction and using PCR, 450 students were chosen, out of them; 20 students were excluded. Throughout the procedure from blood sampling to DNA extraction, 13 samples were lost; 7 were hemolyzed and 6 were inadequate. The remaining samples for genetic test were 417.

After obtaining a signed consent from each participant who met the criteria for selection, participants were asked to fill a simple questionnaire about demographic data, attacks of recurrent abdominal pain, headache, fever, arthralgia, arthritis, chest pain, duration of each symptom and some risk factors such as Consanguinity between parents, Family history of FMF; first or second degree relatives.

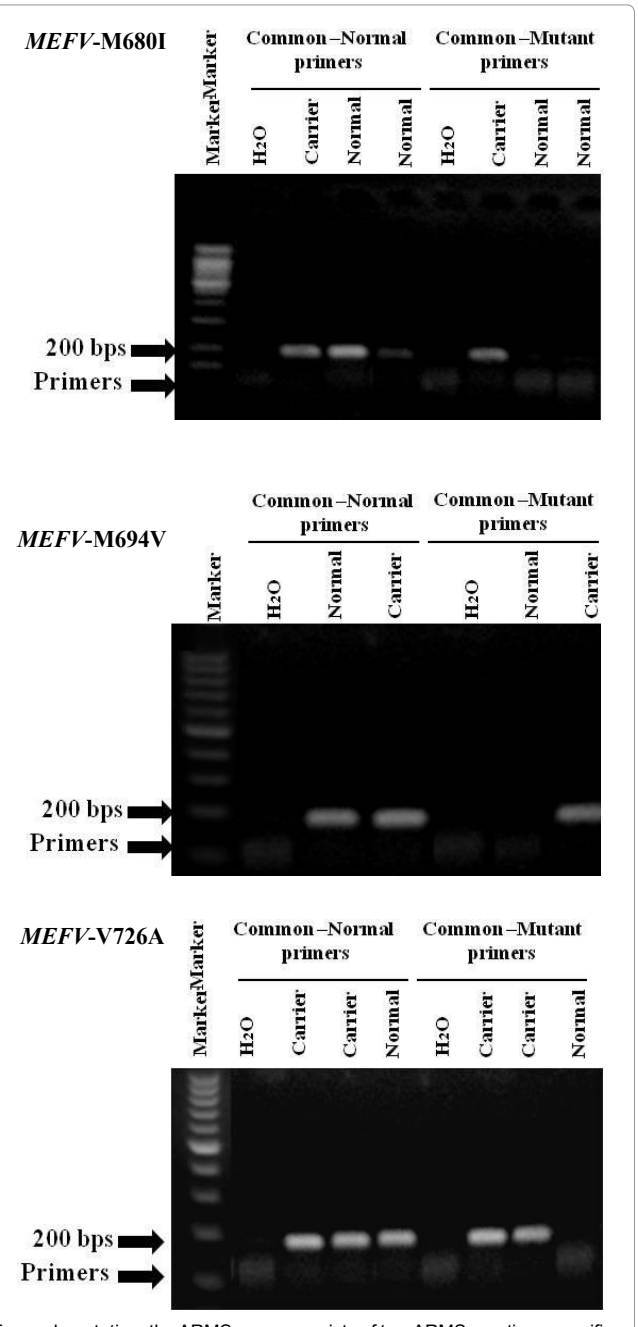
Under aseptic conditions, venous blood of about 3 ml was withdrawn from each participants into EDTA (Ethylene-diamine-tetraacetic acid) containing tube, Then 5 minutes of centrifugation at 3000 rpm was used to separate Serum from the whole blood. Then serum samples were kept at -20°C in sterile micro-tubes until the time of DNA extraction.

DNA extraction and purification

500 µl of WBC cell lyses buffer and 5 µl of proteinase K were added to 200 µl of the buffy coat. The samples were kept at 55°C for 1 hour. DNA was extracted promptly using Phenol-Chloroform Isoamyl Alcohol (P-CIA) protocol [22] and then stored at -20°C.

PCR amplification and gel electrophoresis

The amplification refractory mutation system (ARMS) assay comprises two complementary reactions, each one conducted with the same substrate DNA. One reaction includes an ARMS primer specific for normal DNA sequence, while the second reaction includes a mutant specific primer. The same common primer was used in both reactions as shown in Figure 1. The complementary reaction with normal primer set serves as an internal control for PCR amplification and allows discrimination of homozygous from heterozygous. Mutations were assessed by amplifying the genomic DNA template



For each mutation, the ARMS assay consists of two ARMS reactions specific for the normal or mutant sequences per person. Samples from each study participant were analyzed for each mutation by performing the two reactions. Order of loading was kept from left to right for each reaction. Genotypes ("normal" for homozygous normal, "affected" for homozygous FMF, and "carrier" for heterozygous FMF) were inferred from the combined results of both reactions. Water was added as negative control. The approximate size of the amplified fragments was 200 base pairs (bp); bands migrating anodal to the specifically bands are the residual primers

Figure 1: Detection of three MEFV mutations by amplification refractory nutation system (ARMS) assay.

with three sets of normal and mutant specific ARMS primers designed to selectively amplify the normal or altered sequence of each of three *MEFV* gene mutations. Each set of primers consisted of three oligonucleotides. The primers sequence for each mutation as in Table 1.

The PCR reaction mixture includes total volume of 20 µl containing; 2 µl extracted DNA, Master Mix complex (Reddy Mix PCR, provided by Thermo Scientific), distilled water and 0.08 µl of each primer.

PCR products were analyzed on 2% electrophoresis gel at 120-130 mV for ≈ 1 h to determine the size of amplified fragment. The appropriate positive and negative controls were employed for each run. The positive results were repeated to ensure reproducibility.

The results were read as follows: • The normal: the absence of the 3 mutant lines. • Heterozygous genotypes (carrier): the mutant is present in one allele • homozygous mutant: the mutant is present in the two alleles. In this case the person is a patient of FMF • Compound Heterozygous: the presence of two mutant lines. The prevalence of *MEFV* gene mutations were calculated after excluding positively identified compound heterozygotes [22,23].

Data handling and statistical analysis

Data were entered to the computer and statistical analysis was done using the Statistical Package for Social Sciences (SPSS) version 16. Categorical variables using Chi square test. *P* values were considered statistically significant at $P<0.05$.

Results

A total number of 417 apparently unrelated healthy students of NNU were screened for three common *MEFV* gene mutations worldwide (M694V, M680I (G/C), V726A). Analysis of the demographic distribution of the participants; Parental consanguinity was positive in 72 cases (17.27%), while 17 students (4%) underwent appendectomy. Overall, the prevalence of three common *MEFV* gene mutations in West Bank population among students of NNU was 23.5%. The compound heterozygotes were excluded from the prevalence [23]. The most common mutation was V726A (12.7%), followed by M680I (8.3%), while the least one was M694V (2.4%). The most common compound heterozygous was (M680I/V726A) with five cases and only one of them was symptomatic (recurrent abdominal pain, fever and headache), while two cases were compound heterozygous for (M694V/V726A) with no clinical manifestations. (M694V/M680I) heterozygous was positive in one case that also was asymptomatic. Table 2 shows numbers and frequencies of *MEFV* gene mutations.

Regarding symptoms, 11 carriers suffered from recurrent attacks of abdominal pain, represents (11.4%) of all carriers. On the other hand (8.3%) of carriers complained from recurrent fever that does not subside with antipyretic. There was no significant relation between having *MEFV* gene and suffered from recurrent symptoms (abdominal pain, fever, headache, joint pain). These results are shown in Table 3.

A Chi-square test was used to test the significant relation between *MEFV* gene mutations and gender from the other side. The gender did not show any significant relationship with *MEFV* gene mutations.

Discussion

The prevalence of three common *MEFV* gene mutations (M694V, M680I (G/C), V726A) in The West Bank population among students of NNU was 23.5% with 95% *CI*. To the best of our knowledge, there is no study in Palestine that estimates the prevalence of common *MEFV* gene mutations in apparently healthy population. These results are comparable to almost all the studies carried out on the major affected populations [24], These results also are in accordance with almost all the studies that included Arabic populations [13,25].

In Iran, the number was very close to our study with a prevalence of (25.5%) for the most common *MEFV* gene mutations. The study was done in 2010 in a community sample from couples undergoing pre-marriage screening [26]. Close numbers were reported also in Syria in 2013 with a prevalence of (18.1%) for the five common *MEFV* gene mutations [21]. Another very close numbers to our study were

Table 1: Primer sequences and length designed for ARMS PCR [25].

Mutation	Sequence	Primer Length	Primer Type
V726A	'TGGAGGTTGGAGACAAGACAGCATGGATCC-3-'5	30	Common
	TGGGATCTGGCTGTCACATTGTAAAAGGAGA-'5 'TGCTTCCTG-3	40	Mutant
	TGGGATCTGGCTGTCACATTGTAAAAGGAGA-'5	40	Normal
	'TGCTTCCTA-3		
M694V	'TGACAGCTGTATCATTGTTCTGGGCTCTCCG-3-'5	31	Common
	TCGGGGGAACGCTGGACGCCTGGTACTCATT-'5	40	Mutant
	'TTCCTTCCC-3	40	Normal
	TCGGGGGAACGCTGGACGCCTGGTACTCATT-'5		
	'TTCCTTCCT-3		
M680I	'TTAGACTTGGAAACAAGTGGGAGAGGCTGC-3-'5 ATTATCACCACCCAGTAGCCATTCTCTGGCG-'5 'ACAGAGCG-3	30 39	Common Mutant
	ATTATCACCACCCAGTAGCCATTCTCTGGCG-'5	39	Normal
	'ACAGAGCC-3		

Table 2: Frequencies of *MEFV* genes.

The mutation	Number of carriers	*Frequency (%)
M694V	10	2.4
M680I	34	8.3
V726A	52	12.7
Total	96	23.5

*The calculations of the carrier rate and frequency is carried out under the assumption of that there are no complex alleles (compound heterozygotes)

Table 3: The frequencies of symptoms among carriers and non-carriers.

Symptom		Carrier n (%)	Non-carrier (%)	Total	P value
Abdominal	Yes	11 (21.2)	41 (78.8)	52	0.486
pain	No	92 (25.2)	273 (74.8)	365	
Fever	Yes	8 (24.3)	25 (75.7)	33	0.824
	No	95 (24.6)	289 (74.6)	387	
Headache	Yes	1 (25)	3 (75)	4	0.336
	No	102 (24.7)	311 (75.3)	413	
Joint pain	Yes	1 (14.3)	6 (85.7)	7	0.563
	No	102 (24.9)	308 (75.1)	410	

published in Israel in 2000 with a prevalence of (22%) for the four common *MEFV* gene mutations [23].

Conclusion

In conclusion, the prevalence of the *MEFV* gene mutations was high and similar to neighbor countries such as Syria and Iran. According to these results, genetic counseling can play important role in reducing the number of affected patients, genetic screening in families with affected patients can reduce the risk of developing complications, as amyloidosis, by providing proper prophylactic with colchicine [12].

Limitations of the Study

Prevalence of the five most common mutations worldwide in west bank population was the previous objective of this research, but the limited budget confines the research for just three mutations. Although the sample size was calculated using standard formula, different (or the same) more representative results may be predicted if larger sample number was used. Also, as the research required a blood sample from each participant who fulfills the inclusion criteria, it was difficult to encourage students to participate.

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