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Original Article

Immunogenic Potential of the Mediterranean Fever Gene in Patients with Coronavirus Disease: A Cross-Sectional Study

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Abstract

Background: In December 2019, an outbreak of pneumonia caused by the novel coronavirus disease 2019 (COVID-19) became a pandemic and caused a global health crisis. This study evaluates the immunogenic potential of the Mediterranean fever (*MEFV*) gene in patients with COVID-19.

Methods: A cross-sectional study was conducted from March to April 2020 in various COVID-19 referral centers in Ardabil, Iran. Blood samples of 50 hospitalized patients with confirmed COVID-19 were evaluated for *MEFV* gene mutation using the amplification refractory mutation system polymerase chain reaction (ARMS-PCR) and Sanger sequencing. Statistical analysis was performed using SPSS software, version 22.0.

Results: Mutations of the *MEFV* gene were found in 6 (12%) of the patients. All mutations were heterozygous, and no homozygous or compound heterozygous forms were detected. The total mutant allele frequency was 6% and the carrier rate was 12%. The most common allele of the *MEFV* variant was E148Q, detected in 3 (6%) patients. No mutant variant of the *MEFV* gene was detected in deceased patients. None of the mutation carriers had familial Mediterranean fever (FMF) symptoms or a family history of FMF.

Conclusion: *MEFV* gene mutations may have immunogenic potential in patients with COVID-19.

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Keywords ● COVID-19 ● Familial Mediterranean fever ● *MEFV* gene

What's Known

- The innate immune system plays a role in the immunopathogenesis of coronavirus disease 2019 (COVID-19).
- The importance of the Mediterranean fever (*MEFV*) gene pathway was not studied comprehensively.

What's New

- *MEFV* gene mutations may have immunogenic potential in COVID-19.

Introduction

In December 2019, an outbreak of pneumonia caused by the novel coronavirus disease 2019 (COVID-19) emerged in Wuhan (China) and spread throughout the world.[1] The disease rapidly became a pandemic causing a global health crisis. The main feature of the disease is pulmonary involvement, but the infection can also cause a variety of symptoms including cardiovascular diseases and gastrointestinal disorders.[2-4] However, the pathophysiological aspects of the disease are not yet fully understood. A previous study suggested that the viral infection may trigger an excessive immune response in affected patients.[5] Another study suggested

that cytokine storm syndrome may occur in some patients, leading to extensive tissue damage.[6] Interleukin 6 (IL-6), which plays a key role in a cytokine storm, is produced by activated leukocytes causing the excretion of several other cytokines. The production of these cytokines is mainly triggered to modulate the inflammatory response in order to suppress the infection.[7] Given the role of inflammation in both exacerbation and suppression of the disease, it can be hypothesized that changes in the mechanism of the innate immunity pathway in IL-6 production may result in different clinical features of the disease.[6] Pyrin, one of the key components in innate immunity and inflammation, is expressed in the Mediterranean fever (*MEFV*) gene.[8] The *MEFV* gene is located on the short arm of chromosome 16 at position 13.3 (16p 13.3)[9] and is predominantly expressed in monocytes and granulocytes. These cells play a major role in the pathophysiology of the acute inflammation phase and the development of cytokine storms.[10]

We believe that *MEFV* gene mutations might influence baseline markers of inflammation, suppress inflammatory responses, and affect clinical outcomes. Based on our experience, it seemed that the incidence of COVID-19 was unexpectedly lower among the approximately 600 patients with familial Mediterranean fever (FMF) registered in our database (www.fmfiran.ir). Moreover, published data suggest a slightly lower incidence of COVID-19-associated infection among the population of the Eastern Mediterranean Region, where there is a high frequency of *MEFV* gene mutation carriers. This raises the hypothesis that *MEFV* mutation carriage has a possible protective factor in the COVID-19 pandemic.[11] In line, we hypothesized that *MEFV* gene mutations may alter the clinical response to COVID-19-associated infection. To this end, the present study aimed to explore the potential role of the mutant allele of the *MEFV* gene in COVID-19 patients and to compare its frequency with a healthy population.

Patients and Methods

A cross-sectional study was conducted from March to April 2020 in various COVID-19 referral centers in Ardabil, Iran. Given the subject of our study, this city is of interest due to its proximity to the Eastern Mediterranean Region. Based on a previous study, the prevalence of *MEFV* gene mutations in Northwest Iran was about 25%.[12] Hence, a sample size of 50 was considered adequate to obtain probability type I and type II errors of 0.05 and 0.2, respectively. Using a random number table, 50 patients among all those admitted to the COVID-19 referral centers were recruited.

The study was approved by the Ethics Committee of Ardabil University of Medical Sciences, Ardabil, Iran (code: IR.ARUMS.REC.1399.005). Written informed consent was obtained from the participants. If a patient was unable to sign the form, consent was obtained from first-degree relatives.

DNA Extraction

Peripheral blood samples (10 mL) were collected from the patients using ethylenediaminetetraacetic acid (EDTA) tubes. DNA was isolated using the QIAamp DNA Blood kit (Qiagen, USA) according to the manufacturer's instructions. The amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method was used for E148Q mutation. Detection of the four common MEFV gene mutations was performed using the ARMS-PCR. For each mutation, the ARMS assay consists of two PCR reactions specific for the normal (lanes 17, 22, and 32) and mutant (lanes 10-15 and 18-20) alleles.

Mutation Analysis

The presence of the three most common *MEFV* mutations in exons 2, 3, and 5 (E148Q, P369S, and F479L, respectively) was analyzed using the ARMS-PCR method. However, mutations in exon 10 (M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, and R761H) were analyzed with direct Sanger sequencing using ABI 3130 genetic analyzer (Applied Biosystems and Hitachi Ltd., USA), and aligner software version 9.0.1 (CodonCode Corporation, USA).

Statistical Analysis

Data were analyzed using SPSS software, version 22.0 (IBM Corp., Armonk, N.Y., USA). The Chi square test and Fisher's exact test were used to compare data between different categories. The non-parametric method was used to test for the difference in proportions. $P<0.05$ was considered statistically significant.

Results

Of the 50 patients, 23 (46%) and 27 (53%) were male and female, respectively. The mean age of the patients was 54.22±20.3 years and was normally distributed. *MEFV* gene variants were found in 6 (12%) of the patients, which was significantly lower than the reported 25% carrier rate common in the healthy population

Table 1: Clinical findings in COVID-19† patients

Clinical findings		Carrier (%)	Non-carrier (%)	Total (%)	P value
Manifestation	Fever	4 (66.7%)	34 (77.3%)	38 (76%)	0.05
	Cough	4 (66.7%)	28 (63.6%)	32 (64%)	0.05
	Dyspnea	1 (16.7%)	14 (31.8%)	15 (30%)	0.05
	Myalgia	3 (50%)	19 (42.3%)	22 (44%)	0.05
	Nausea	1 (16.7%)	9 (20.5%)	10 (20%)	0.05
	Vomiting	0	4 (9.1%)	4 (8%)	0.05
	Lymphopenia	6 (100%)	34 (77.3%)	40 (80%)	0.05
Outcome	Death	0	6 (13.6%)	6 (12%)	0.05
	Hypoxia ($SatO_2$<85%)	2 (33.8%)	8 (18.2%)	19 (38%)	0.05
	Elevated LDH levels (U/L)	4 (66.7%)	34 (77.3%)	38 (76%)	0.05
	Elevated ESR levels* (mm/hr)	5 (83.3%)	33 (80.5%)	38 (80.9%)	0.05

*Missing data in three patients, ESR: Erythrocyte sedimentation rate, LDH: Lactic dehydrogenase

in Northwest Iran (P=0.025).[12] All mutations were heterozygous, and no homozygous or compound heterozygous forms were detected. The total mutant allele frequency was 6%. The most common allele of the *MEFV* variant was E148Q, detected in 3 (6%) patients. The variants A774S, V726A, and P369S were found in three patients. There were no variants of the *MEFV* gene among the deceased patients, but the difference between the deceased, and alive patients was not statistically significant. Among the severely infected, two patients had E148Q and A744S mutations. None of the mutation carriers had FMF symptoms or a family history of FMF (table 1).

Discussion

In this study, a significantly lower incidence of *MEFV* gene variants was found among COVID-19 patients compared to the healthy population in Northwest Iran. The protective effect of these variants against some infectious diseases was previously proposed. It was suggested that heterozygous carriers might have a higher resistance to tuberculosis and brucellosis.[13, 14]

The fact that many COVID-19 patients are clinically asymptomatic indicates that the immune system may have the ability to defeat the coronavirus.[15] While this ability involves the immune system as a whole, the innate immune response as a trigger for inflammation was attributed to immunogenetics and cytokine storms.[6, 7] There is significant evidence that hyperactive innate immunity causes acute lung injury in patients requiring hospitalization.[16] IL-6 is secreted from macrophages, key cells in innate immunity, initiates an inflammatory cascade and the release of several cytokines (including IL-1), resulting in an increased population of monocytes.[17-19] IL-6 increases inflammation by the subsequent release of various immune mediators. This response may cause a state of hyperactivity, and therefore it is suggested that IL-6 levels correlate with the severity of the disease and mortality.[20]

Pyrin, also known as marenostrin, is encoded by the *MEFV* gene[8] and plays a key role in apoptotic and inflammatory signaling pathways. Pyrin modulates caspase-1 and IL-1β activation and may exert pro-inflammatory[21-23] or anti-inflammatory effects.[8, 24] Stimulation of monocytes with pro-inflammatory agents (e.g., bacterial lipopolysaccharide, interferon-γ, and tumor necrosis factor-a) induces the expression of *MEFV* gene, suggesting a role in inflammatory signaling cascades.[25, 26] Due to its role in inducing IL-1β, alteration in pyrin function as a result of *MEFV* gene mutations may influence clinical response to viral infections, particularly infection due to COVID-19. This hypothesis is supported in recent clinical studies suggesting that non-selective NLRP3 inhibition by colchicine in COVID-19 patients with FMF may influence the clinical presentation of the infection.[27-29]

The prevalence of COVID-19 cases per million people among Caucasians and the Eastern Mediterranean population were reported to be relatively lower than other populations (www.news.google.com/covid19/map). Although these findings are affected by several contributing factors, the immunogenetics of *MEFV* gene expression may also play a role in the epidemiological aspects of the disease. Ethnicity is associated with susceptibility to infection,[30] and ethnic similarity between the populations of Eastern Mediterranean countries could be the reason for a relatively high prevalence of *MEFV* gene variants and consequently FMF disease.

A mutant allele frequency of up to 15.6% and a carrier rate of up to 20%-25% were reported in the healthy population of different Eastern Mediterranean countries.[12, 31-33] Interestingly,

in our COVID-19 patients, the allele frequency of the *MEFV* variant was only 6% with no homozygous or compound heterozygous mutation and a carrier rate of 12%. This prevalence is significantly lower than the healthy population in Northwest Iran,[12] suggesting a probable protective role of *MEFV* gene variants in COVID-19[+] patients.[31] Interestingly, none of our COVID-19 patients with a variant of *MEFV* gene died because of the disease.

Our results were not statistically significant due to the small sample size, but the difference was clinically interesting. These variants may affect mortality in COVID-19 patients and may explain the high mortality rate among Hispanics, Western Europeans, African-Americans, and Black Britons (www.news.google.com/covid19/map). The prevalence of *MEFV* gene variants in these groups of people is lower than in the healthy population in the Eastern Mediterranean Region.[32-34]

Our research provides valuable information, however, further studies on different ethnic groups are required to substantiate our findings. The main limitations of our study are the small sample size and the single-center design undermining the generalizability of the data to other COVID-19 variants.

Conclusion

Given the high carrier frequency of *MEFV* gene variants in the Eastern Mediterranean Region and a significantly lower prevalence of these variants in COVID-19 patients, it seems that *MEFV* variants may have immunogenic potential and reduce the incidence rate, morbidity, and even mortality of the disease.

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Authors' Contribution

F.S and F.A: Concept and design of the study, diagnosis and management of patients, B.D: Genetic analysis, F.P: Data analysis, R.M: Diagnosis and management of patients, E.Sh: Data collecting. All authors participated in writing the manuscript. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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