The role of MEFV gene in COVID 19 disease, as a protective factor

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Abstract

Background

In early 2020, an outbreak of pneumonia caused by a novel coronavirus became pandemic. This study evaluates the potential immune-genetically role of *MEFV* gene mutations in COVID 19 patients.

Methods

50 COVID 19 PCR positive patients who were hospitalized in COVID 19 referral centers between 1st of March to 30th of April in 2020 were evaluated for *MEFV* gene mutations using ARMS PCR and Sanger sequencing.

Results

MEFV gene mutations were found in 6 (12%) of the patients. No homozygote or compound heterozygote forms were detected. The total mutant allele frequency was 6%. The carrier rate was 12% which is significantly lower than previously studied rate of 25%. The most common *MEFV* variant was E148Q in 3 (6%). There was no mutant variant of *MEFV* gene among the expired patients. None of the *MEFV* gene mutant patients had FMF symptoms or positive family history of FMF disease.

Conclusion

Considering the high carrier rate of *MEFV* gene mutations in the eastern Mediterranean region and a significantly lower prevalence of these mutations in COVID 19 patients, it seems that *MEFV* gene mutations may have a protective role in incidence of the disease.

Key Point

MEFV gene mutations may have a protective role in morbidity and mortality of COVID 19 patients.

Background

In December 2019, an outbreak of pneumonia caused by a novel coronavirus occurred in Wuhan, Hubei province, and spread throughout the world (1). The disease rapidly became pandemic which caused a global crisis. The main feature of the disease is pulmonary involvement but the infection can also develop a variety of symptoms including cardiovascular and gastrointestinal ones (2–4). The pathophysiological aspects of the disease are not completely clear yet. Many performed studies suggest that the viral infection may cause the production of an excessive immune reaction in these patients (5). Accumulating data suggests that in some cases a reaction named "cytokine storm syndrome" may

happen which causes an extensive tissue damage (6). IL-6 which plays the main role in cytokine storm, is produced by activated leukocytes causing excretion of several other cytokines subsequently. On the other hand, the production of these cytokines is mainly triggered in order to develop an inflammation to suppress the infection (7). Considering the role of inflammation in both exacerbation and suppression of the disease it can be hypothesized that altered mechanisms of innate immunity pathway as a main role in IL-6 production may result in different clinical features of the disease.

Pyrin is one of the key components in innate immunity and inflammation which is expressed from *MEFV* gene (8). The *MEFV* gene is located on the short arm of chromosome 16 at position 13.3(16p 13.3) (9). *MEFV* gene is predominantly expressed in monocytes and granulocytes. These cells have major roles in the pathophysiology of inflammation at the acute phase and developing the cytokines storm (10).

It has been proposed that *MEFV* mutations might increase the baseline of inflammation, induce the development of inflammatory process, and affect the clinical course of this new disease.

Furthermore, based on our experience it seemed that incidence of COVID 19 infection was unexpectedly lower among the approximately 600 registered FMF patients in our database (www.fmfiran.ir). Also, published statistics suggest a slightly lower COVID-19 infection incidence among populations of eastern Mediterranean region in whom, *MEFV* gene mutation carriage is high. This raises a hypothesis of *MEFV* mutation carriage being a protective factor for COVID-19 pandemic (11). Based on this finding, we hypothesized that *MEFV* gene mutations may alter the clinical response to COVID 19 infection, as well as the *MEFV* gene role in inflammatory processes. To clarify this potential role of the *MEFV* gene variants in COVID 19 patients and comparing its frequency with general population this study has been conducted.

Methods

Patients

On the basis of our previously published study, the prevalence of *MEFV* gene mutations was relatively equal to 25% in the region (12). Considering this prevalence we calculated that a sample size of 50 would be adequate with probable first and second type errors equal to 0.05 and 0.2, respectively. Then the patients were selected using random number table among all patients admitted to referral COVID 19 centers of northwest of Iran (Ardabil) which is near to eastern Mediterranean area.

DNA extraction

First, 10 mL of peripheral blood was collected from each patient into EDTA-anticoagulated tubes. DNA was extracted from the samples using QIAamp DNA Blood Isolation kit (Qiagen GmbH) by standard methods.

Mutation analysis

The presence of the three most common *MEFV* mutations on exons 2, 3 & 5 was determined using amplification refractory mutation system polymerase chain reaction (ARMS). The noted mutations were E148Q, P369S & F479L, respectively. Meanwhile, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, and R761H mutations which were relative to exon 10 were analyzed by direct sanger sequencing using ABI 3130 genetic analyzer and were read by codon code software.

Statistical analysis

Statistical analysis was performed using SPSSv22. Comparison between the different categories was assessed using chi-square or Fisher's exact test depending on the situation. The nonparametric proportion test was done for further analysis. A P-Value lower than 0.05 was accepted as statistically significant.

Results

From enrolled 50 patients, 23 (46%) were male and 27 (53%) were female. Mean age of the patients was 54.22 ± 20.3 years and it was distributed normally. *MEFV* gene variants were found in 6 (12%), which was significantly lower than the expected carrier rate of 25% in normal population of northwest of Iran ($P = 0.025$) (12). All mutations were heterozygous and no homozygote or compound heterozygote forms were detected. The total mutant allele frequency was 6%. The most common *MEFV* variant was E148Q in 3 (6%) and 3 patients had a variant of A774S, V726A and P369S. There was no mutant variant of *MEFV* gene among the expired patients but the difference between two groups was not statistically significant. Among the severely infected, two patients had mutations of E148Q and A744S. None of the mutation carriers had FMF symptoms or positive family history of FMF disease. (Table 1)

Table 1
_ Clinical findings of COVID 19 patients

		Carrier (%)	Non Carrier (%)	Total (%)	p-Value
Manifestations	Fever	4 (66.7%)	34 (77.3%)	38 (76%)	NS
	Cough	4 (66.7%)	28 (63.6%)	32 (64%)	NS
	Dyspnea	1 (16.7%)	14 (31.8%)	15 (30%)	NS
	Myalgia	3 (50%)	19 (42.3%)	22 (44%)	NS
	Nausea	1 (16.7%)	9 (20.5%)	10 (20%)	NS
	Vomiting	0	4 (9.1%)	4 (8%)	NS
Lymphopenia		6 (100%)	34 (77.3%)	40 (80%)	NS
Outcome	Death	0	6 (13.6%)	6 (12%)	NS
Hypoxia (SatO2 < 85%)		2 (33.8%)	8 (18.2%)	19 (38%)	NS
Elevated LDH levels		4 (66.7%)	34 (77.3%)	38 (76%)	NS
Elevated ESR levels*		5 (83.3%)	33 (80.5%)	38 (80.9%)	NS
* Missing data in 3 patients					

Discussion

In this study, we found a significantly lower incidence of MEFV gene mutation carriage rate among patients with COVID-19 infection in comparison to the normal population in the region which was in line with our primary hypothesis. The idea of MEFV gene mutations protective role against infectious diseases or other environment factors is been proposed previously due to the preserved status of high MEFV gene mutations throughout centuries. For example it's suggested that heterozygote carriers may have a higher resistance against tuberculosis and brucellosis (13, 14), however these hypotheses are not proved due to lack of adequate studies.

Many COVID 19 exposed people clear the virus without presenting any symptoms. This fact suggests that the immune system may have the capability to defeat the virus (15). Although this capability includes whole immune systems, innate immunity as a potential part of inflammation has been emphasized in COVID 19 immune-pathogenesis and it's cytokines storm.

Tissue-resident macrophages are one of the first main leukocytes in the process of triggering the acute inflammation in response to damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) during early pulmonary viral infections including COVID 19 (5, 16). There are remarkable evidence of innate immune hyperactivity in driving the acute lung injury that defines patients with requirement of hospitalization (5). These macrophages initiate an inflammatory cascade resulting in

release of several cytokines including IL-1 which enhances the endothelial activation and continuation of acute inflammatory response and recruitment of neutrophils and cytotoxic T cells (5, 17).

IL-6 is another key cytokine, released from macrophages as a key cell in innate immunity and increased population of monocytes, partly in response to the priorly secreted IL-1 (18, 19). IL-6 results in an increase of the inflammation by subsequent release of various immune mediators (20). This response may cause a hyperactive state as evidence suggest IL-6 levels correlate with severity of the disease and its mortality (20).

Considering the role of IL-1 in triggering such a state, the preliminary events leading to the secretion of IL-1 may influence the noted cascade. Pyrin is considered to be one of the precedential agents (21). Pyrin (also known as marenostin) is encoded by MEFV gene (8). It plays a key role in apoptotic and inflammatory signaling pathways. Pyrin modulates caspase-1 and IL-1 β activation exerting both pro-inflammatory (21–23) or anti-inflammatory mechanisms (8, 24). There are several evidence suggesting a pro-inflammatory function for Pyrin as well as its anti-inflammatory functions depending on the experiment. For example, Stimulation of monocytes with pro-inflammatory agents such as bacterial lipopolysaccharide (LPS), interferon- γ and tumor necrosis factor- α (TNF- α) induces the expression of MEFV, suggesting a role in the inflammatory signal cascades (25). Due to its role in inducing IL-1 β , alteration in Pyrin function through MEFV gene mutations may influence the upcoming events in the clinical response to the viral infections, particularly, COVID 19 infection. This hypothesis is strengthened while looking at the recent findings in clinical trials which suggest that the non-selective NLRP3 inhibition by colchicine may enhance the clinical presentation of COVID 19 infection, considering the role of NLRP3 in formation of IL-1 β from pro-IL-1 β (26).

Meanwhile, the worldwide distribution shows that the prevalence of COVID 19 infection per one million individuals is relatively lower in the Caucasians and population of eastern Mediterranean region (www.news.google.com/covid19/map). Although this epidemiologic perspective is under several influential factors such as different screening methods, the genetic basis of MEFV gene expression may also have a key role in the epidemiologic aspects of the disease.

Considering that ethnic differences of populations affect the presentation of infection diseases (27), there are some similarities among the Mediterranean populations. These regions have a relatively high prevalence of *MEFV* gene mutations and consequently, FMF disease. For example a mutant allele frequency up to 15.6% and a carrier rate up to 20–25% is reported among normal population of different Mediterranean people (12, 28–30). Interestingly, in our sample of hospitalized COVID 19 infected patients, the mutant MEFV allele frequency was only 6% with no homozygote and compound heterozygous form and a carrier rate of 12%. This prevalence seem to be significantly lower than the normal population of the region (12), suggesting a probable protective role of MEFV gene mutations in COVID 19 infection. The idea of its protective role has been raised not only by this study, but also other report in the region (31).

Interestingly, none of the patients who had a mutant variant of *MEFV* gene died in the course of COVID 19 infection. Although due to small sample it was not statistically meaningful, this difference was clinically

significant and requires further studies with a larger sample size. These mutations may affect the mortality of COVID 19 patients and it may explain the high mortality rate among Hispanic, western European, black Americans, and British COVID 19 patients (www.news.google.com/covid19/map) in whom, the prevalence of *MEFV* gene mutations are lower than the normal population of eastern Mediterranean region (32–34). Although we think this finding as a first study in COVID disease is very interesting, however, the limitation of small size of patients necessitates its rework in a larger sample sizes and different ethnic groups.

Conclusion

Considering the high carrier rate of *MEFV* gene alleles in Mediterranean region and a significantly lower prevalence of these mutations in COVID 19 infected patients, it seems that *MEFV* gene mutations may have a protective role in incidence of the disease and they may also have a protective effect on COVID 19 mortality rate.

Abbreviations

MEFV
Mediterranean fever gene
FMF
Familial Mediterranean fever

Declarations

Ethics approval and consent to participate:

This study has been supported by **Domestic Committee of faculty of medicine** Ardabil University of Medical Sciences (ARUMS) and has been approved by number: IR.ARUMS.REC.1399.005

Written informed consent was obtained from all participants.

Consent for publication:

Authors taken written informed consent, to do of this work.

Availability of data and material:

Not Applicable

Competing interests:

The authors declare that they have no competing interests.

Conflict of Interest:

There is not any conflict of interest in this study.

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Authors Contributions:

All authors have read and approved the manuscript and they contribute as :

F.S& F.A: Designed and performed the study and co-wrote the paper

B.D: Performed genetic analysis

F.P: Performed epidemiological and statistical analyses.

R.M & F.A& F.S: worked in diagnosis and management of patients.

E.Sh: Collecting data and co-wrote the paper

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Availability of data and material:

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