Research Article

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Innate Immune role in Refractory Epilepsy as an auto-inflammatory immunogenic factor through MEFV gene Variant Alleles.

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ABSTRACT

Background : About 30% of epilepsies are refractory to anti-epileptic drugs, many studies have suggested that innate immune system and inflammatory processes can have a potential role in pathophysiology of refractory epilepsy (RE). *MEFV* gene plays a major role in auto-inflammatory disorders and innate immune system. The aim of this study is to determine the possible role of *MEFV* gene mutations in children with refractory epilepsy.
Method and Materials : The peripheral blood of 15 patients who had RE were collected and the samples screened for the 12 common pathogenic variants alleles of *MEFV* gene using ARMS-PCR and Sanger Sequencing method, and compared with the results of healthy control group.
Results : The patients' group included 7 girls (46.7%) and 8 boys (53.3%). The mean age of patients was 9.33 years and at the onset of seizures was 1.66 years. None of the patients showed these common pathogenic variants alleles.
Conclusion : This study showed despite the possible and potential role of auto-inflammatory processes and innate immune system involvement in RE, there is no association between activation of innate immune pathway by *MEFV* gene variants alleles as an auto-inflammatory immunogenic factor.

Keywords : Refractory Epilepsy (RE), *MEFV* Gene, auto inflammatory

INTRODUCTION

Epilepsy is the most common neurological condition among all age groups and affects more than 50 million people all around the world [1]. Epilepsy is defined as a condition characterized by unprovoked recurrent (two or more) epileptic seizures [2, 3]. The incidence of epilepsy in children is approximately 50 per 100,000 individuals per year [4].
Epilepsy can have a negative impact on patient's life in so many ways such as a combination of physical and mental consequences of seizures, effects on social and familial position and psychological outcomes of both and a reduced quality of life [5-7]. Epilepsy can have different causes such as genetic mutations, metabolic disorders, developmental disorders and neurological trauma [8]. Despite all the advances in the field of anti-epileptic drugs (AED) about 30% of patients with newly

diagnosed epilepsy are resistant to treatment [9].

Until this time there hasn't been a unanimous and precise definition of Refractory Epilepsy (RE) [10], but a definition that is generally accepted was proposed by ILEA (International League Against Epilepsy): "failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" [11]. The exact pathophysiology of RE isn't fully understood yet [12], but some studies have suggested that inflammatory processes and innate immune system may have a key role in epileptogenesis, these processes are responsible for the disruption of Blood Brain Barrier (BBB) and the activation of immune cells in brain [12-14]. Some of the used AEDs have anti-inflammatory effects [15-17]. The response to corticosteroid drugs in refractory epilepsy was promising [12, 18, and 19]. In 2011 a study showed that the IL-6 levels in people with epilepsy and intellectual disability were significantly higher than the controls [20]. Recently a study showed that IL-1 blocking drugs such as Anakinra can have a positive effect on treating refractory epilepsy [21]. So it can be concluded that inflammation by innate immune system might have a potential role in etiology and pathogeneses of RE.

MEFV (Mediterranean fever) is a human gene that encodes a protein called Pyrin (also known as Marenostrin). Mutation in *MEFV* can cause Familial Mediterranean fever (FMF) that is the most prevalent hereditary auto-inflammatory syndrome [22]. Increasing evidence show that *MEFV* gene might have a role in conditions other than FMF [23]. Some studies have revealed that *MEFV* mutations are associated with different types of systemic vasculitis such as Behcet's disease, Henoch-Schonlein purpura, and Polyarteritis Nodosa. This suggests that the potential role of *MEFV* gene mutations in broader spectrum of medical conditions and indicates that these mutations might increase the baseline of inflammation and affect the clinical features of inflammatory diseases [24]. According to latest studies there might be a connection between *MEFV* gene mutations and neurologic conditions such as childhood Multiple Sclerosis (MS) and Migraines [25, 26].

Given the potential role of inflammatory processes and innate immune system in epileptogenesis and the role of *MEFV* gene in innate immunity by production of IL-1 and IL-6 and the high prevalence of *MEFV* mutations in northwest of Iran [27] we investigated the possible role of common variants alleles of *MEFV* gene mutations in patients with RE.

METHODS

Patient group included 15 RE patients in Neurology clinic of Bouali Children's Hospital of Ardabil between the year of 2020-2021. All of them were under 18 years old and had a negative history of FMF symptoms in themselves and in their first-degree family. None of the patients had a known metabolic, inflammatory and or traumatic reason for their seizures. Based on our previously published work, the prevalence of *MEFV* gene mutations variants alleles were relatively equal to 25% in normal population of this area [28].

Blood samples were collected from the participants and the samples were screened for the 12 common pathogenic variants of *MEFV* gene (E148Q, F479L, P369S, I692del, M680I (G/C), M680I (G/A), M694V, V726A, A 744S, M694I, K695R, R761H).

DNA extraction

First, 10 mL of peripheral blood was collected from each patient into EDTA-ant coagulated tubes. DNA was extracted from the samples using QIAamp DNA Blood Isolation kit (Qiagen GmbH) by standard methods. ARMS-PCR result for E148Q mutation. Detection of four common *MEFV* gene mutations by ARMSPCR. For each mutation, the ARMS assay consists of two PCR reactions specific for the normal and mutant alleles. Lane17, 22, and 32 are reactions for mutant alleles and lanes 10, 11, 12, 13,15,18,19 and 20 are reactions for normal alleles.

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.

All data were analyzed by simple statistical method. And p value< 0.5 was consider meaningful.

The study is complaint with the Helsinki Declaration and was approved by domestic ethics committee of faculty of medicine ARUMS (Ardabil University of Medical Sciences), approval number: IR.ARUMS.REC.1399.567. Informed consent was obtained from all parents' individual participants Included in the study.

RESULTS

This study included 7 girls (46.7%) and 8 boys (53.3%) as patient group. The youngest patient was 3 years old and the oldest one was 16, the median age was 11 years. The earliest age of onset was 3 months and the latest was 8.5 years, the median age at the onset was 6 months. Regarding *MEFV* gene analysis none of the patients showed mutations.

The prevalence of these mutations in RE patients is significantly and meaningfully lower than the normal population which is 25% (P value< 0.001).

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Among 224 healthy control cases, 113 (50.4%) were male and 111 (49.6%) females. There were *MEFV* variants alleles in 57 patients (25%): 28 were male (49.1%) and 29 were female (50.9%). The most frequent variants were E148Q (18.3%), followed by P369S (3.1%), V726A (2.2%), A744S (1.3%), and F479L, M694V, and R761H (0.8%), and eventually K695R (0.4%), respectively.

DISCUSSION

Although many new AEDs (Anti-epileptic Drugs) have been introduced since 1990s, about 30% of patients with newly diagnosed epilepsy are resistant to treatment. [9] The pathophysiology of RE is still not completely clear; however, It seems that the mechanisms of refractory epilepsy are most likely multifactorial and a result of interaction between environmental, genetic, as well as disease- and drug-related factors [29, 30] In 1995, Tishler et al suggested that the overexpression of P-glycoprotein (P-gp) at the blood–brain barrier in epilepsy causes decreasing in AED brain uptake and results in RE [31]. Some studies suggest that refractoriness is a result of alterations in the properties of AED targets, like structural changes in voltage-gated ion channels and neurotransmitter receptors [32, 33].

Recently the role of innate immune system and inflammatory process in epileptogenesis and RE have been a field of interest for researchers and scientists [34].

The CSF and serum of patients with chronic epilepsy showed increased levels of pro-inflammatory cytokines [35-37]. IL-1B, IL-2 and IL-6 that usually exist in low level at brain are increased after seizures [38]. In a clinical study about patients with prolonged febrile seizures the levels of IL-IB, IL-6 and TNF-α (tumor necrosis factor) were elevated [38]. Furthermore, some studies showed that the expression of mRANs associated with IL-1B, IL-6, TNF-α, TGF-β1 (transforming growth factor- beta 1) and VEGF (vascular endothelial growth factor) after seizure is increased in hippocampus [38-40, and 41].

These evidences provide the possible role for involvement of neuro-inflammation in epileptogenesis. A study suggested that epileptogenesis is characterized by complex unregulated inflammatory molecules and pathways found in both the nervous system and systemic tissue [42].

Given the potential role of immune system in mediating the pathophysiology of epilepsy, targeting this system, especially the pro-convulsant cytokines, has been suggested as a potential therapeutic strategy in drug resistant epilepsies [43]. A study showed that the usage of IL-1 blockers such as Anakinra and Canakinumab may have a positive effect on patients with RE [23]. Another study showed that Anakinra reduces the frequency, duration and neuronal loss of spontaneous recurrent seizures [44]. Anakinra also has been used for the treatment of Colchicine resistant FMF [45, 46].

MEFV mutations result in production of mutated pyrin and thus uncontrolled and prolonged inflammations, although there is a well-established link between pyrin mutations and FMF, to this time the exact role of pyrin and how mutated pyrin promotes disease isn't fully understood.

Some evidence suggesting that pyrin acts as a positive or negative regulator of caspase-1 activation which is a cysteine protease responsible for activation of pre-inflammatory cytokines Interleukin-1Beta (IL-1B) and IL-18 [47]. In agreement with an anti-inflammatory role of pyrin, the downregulation of pyrin by siRNA in human acute monocytic leukaemia cell line-1 cells increase the production of IL-1B [45].

In a study about the neurological manifestations of FMF, 27.3% of patients were suffering from epilepsy that was significantly higher than the general population [48].

In spite of these findings in favor of more neurological and seizure prevalence among FMF patients, this study's results of null *MEFV* mutations in patients with RE, can suggest that inflammatory processes, inflammasome assembly and activation of innate immune pathway due to *MEFV* mutations probably don't have a significant role in pathophysiology of RE.

Small group of patients is the limitation of this study and to determine the significant of finding a study with larger sample size and investigation of all known mutations is required.

CONCLUSION

Despite the possible and potential role of auto-inflammatory processes and innate immune system involvement in RE there is no association between activation of innate immune pathway by *MEFV* gene as an auto-inflammatory immunogenic factor.

Abbreviations

FMF: Familial Mediterranean fever;
MEFV: Mediterranean fever Gene;
RE: Refractory Epilepsia

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None.

Authors' contributions

FA: carried out the management and diagnosis of patients. MM&FS: Participated in the design of the study and performed the final copy of manuscript. BD. performed genetic analysis. MM (Maryam) collected all data and performed draft copy of manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current

study are available in the SALEHZADEH F. repository, please contact first author for data request,

Ethics approval and consent to participate

The study is compliant with the Helsinki Declaration and was approved by domestic ethics committee of faculty of medicine ARUMS (Ardabil University of Medical Sciences), approval number: IR.ARUMS.REC.1399.567. Informed consent was obtained from all parents' individual participants Included in the study.

Consent for publication

Not applicable.

Declaration of Conflicting Interests

The Authors declare that there is no conflict of interest.

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