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An Analysis on Pyrin E148Q Mutations in Patients with Refractory Rheumatoid Arthritis

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Abstract

Objective: Rheumatoid arthritis (RA) is an autoimmune disease with lifelong disability in adults. Given the high prevalence of Mediterranean fever and its overlapping and modulatory effects on autoimmune diseases, we analyzed molecular mutation in exon 2 of *MEFV* gene in patients with refractory RA and healthy subjects as a control group.

Methods: Thirty four patients with refractory RA and fifty healthy controls were conducted in this study and their DNA samples were analyzed. We studied the most common exon 2 variation on *MEFV* gene which known as E148Q. At the end of this study, we compared the results of both groups.

Results: The mean age of patients with refractory RA were 43.8 ± 7.7 years and disease duration was 49.8 ± 7.36 months. The mean of DAS 28 (Disease Activity score) of patients with refractory RA was 4.2 ± 0.51. The mean ages of healthy control cases were 45.4 ± 8.27 years. The mutation frequency of exon 2 *MEFV* variant was 6 (17.6%) of 34 and 5 (10%) of 50 in the refractory RA patients and healthy subjects, respectively. According to results no significant difference were observed between two groups (P=0.405).

Conclusion: Overall mutation rate of E148Q gene has not increased in patients with refractory rheumatoid arthritis and this mutation possibly has no effect on disease severity.

Keywords: Refractory rheumatoid arthritis; *MEFV* gene; Mutation; Exon 2

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease which symmetrically affects joints [1]. The incidence and prevalence of RA varies based on geographic conditions but, RA affects 0.5-1% of adult populations worldwide [2]. Its prevalence in rural and urban area of Iran is about 0.19 and 0.51 percent, respectively [3,4]. It has been recognized that genetic factors play rules in occurrence of RA as well as its severity [1,2]. The human leukocyte antigen (HLA) DRB1 alleles and Tumor necrosis factor (TNF) alleles are two important genetic factors which contributed to development of RA [5,6]. There are other non-MHC genes which also attributed to be part of RA [6].

Despite inhibition of inflammation and autoimmune activation with effective drugs such as methotrexate and biologic disease modifying antirheumatic drugs (DMARDs), some of patients remain refractory to treatment [7,8].

One important genetic factor that may have roles in severe and drug resistance of inflammatory arthritis is a recently recognized Mediterranean fever (*MEFV*) gene mutation [1,9,10]. *MEFV* gene is located on chromosome 16p13 and comprises 10 exons and 781 codons and produces a protein, named, pyrin or Marenostrin [1,11,12]. It proposed that, the protein has a level of inhibitory effects on inflammation at the level of leucocyte cytoskeletal organization on polymorphonuclear cells and monocytes [1,11,13-15]. The carrier rate of *MEFV* gene in Mediterranean and middle eastern populations is about one in three to one in five [16].

Almost thirty six mutations have been introduced in exons 1, 2, 3, 5, 9 and 10 in the *MEFV* gene [11]. Two apparent mutational `hot spots' in *MEFV* gene exist mostly in exon 10 and exons 2 [11]. Four of five common mutations-M694V, V726A, M680I, and M694I-have been located in exon 10 and one E148Q in identified in exon 2 [11,16]. The distribution of the four most common *MEFV* mutations among healthy individuals (M694V 29%, V726A 16%, M680I 2% and E148Q 53%) was significantly different (P<0.003) [17].

Some studies showed an association between inflammatory arthritis and *MEFV* gene mutations [1,10,13,18-20]. Koca et al. in their research also presented the same result. They found that *MEFV* mutations prevalence is high in RA patients appear to be an aggravating factor for the severity of RA [13]. In the study, carried out by Rabinovich et al. the results were similar and they report the possibility of the modifying effect of the *MEFV* gene mutations on the expression of certain inflammatory diseases including RA. The study also showed that the disease severity was much more in patients who were carrier of mutated gene compared with non-carrier group and most of mutations were in exon 2 [1]. There are some other studies that mark the

modifying effect of MEFV mutations on inflammatory arthritis [10,14,21].

According to this studies and because *MEFV* mutations were linked to rheumatoid arthritis and may have roles in its severity, it is important to investigate the impact of this genotype on patients with refractory RA. We adopted a case-control design, to compare the exon 2 *MEFV* gene mutations frequency between patients with severe refractory RA and healthy peoples, and to compare associations between genotype and disease activity score (DAS 28) in patients with severe refractory RA.

Material and Methods

This study was carried out in the Emam Reza hospital, Faculty of Medicine, Tabriz University of Medical Sciences. Ethics approval was obtained for this study under Tabriz University of medical science Ethics Committee guide-lines, department of internal medicine (number 92/3-8/30), and conducted according to the International guidelines for medical Research. Experimental procedures were carried out using patient blood samples. Patients invited voluntarily without any cost to take part in this study. We obtained the patients written informed consent to publish the material. Consent forms are completed either by patient or medical staff after elucidation of the sample collection procedure and purposes.

Selection of patients

Thirty eight consecutive patients with severe refractory RA which were diagnosed according to Rheumatology/European League Against Rheumatism Collaborative criteria participated in the study [22]. Patients who have not been in remission despite treatment with Methotrexate 25 mg weekly, Prednisolone 7.5 mg daily, Hydroxychloroquine 400 mg daily and sulfasalazine 1500 mg daily and had DAS 28 more than 2.6, mentioned as refractive RA patients [2]. For comparison, fifty healthy peoples were also included in the study as control group. Four patients with severe refractory RA were excluded because of poor quality of their samples. We obtained the patients written informed consent to publish the material.

Sample preparation and DNA isolation

For this aim, 2 ml of whole blood sample is collected from each participant in a tube including EDTA as anticoagulant under sterile conditions. EDTA-anticoagulant blood samples were centrifuged by 2500 rpm and transferred to 1.5 ml sterile tubes using RNAse and DNAse free tips. According to the manufacturer's instructions, Genomic DNA was extracted from buffy coat section of blood using the QIAamp DNA blood Mini kit (50) from QIAGEN (Cat no. 51304). The quality and quantity of extracted DNA was measured by Nano-drop (Thermo Scientific). Extracted genomic DNA was stored in -80 for analyzing. Specific primers were designed for DNA amplification of *MEFV* gene exon 2, protein coding region using PCR and products were electrophoresed to visualize the DNA bands on agarose gel. One common *MEFV* gene mutations -E148Q- were covered by primers.

Sequencing

MEFV (exon 2) Sequencing was undertaken in both forward and reverse directions using the sequencing primers. Sequencing reactions were prepared for DNA fragments and products were sent for sequencing.

MEFV forward sequence 5′-TTACTGGGAGGTGGAGGTTG-3′ and *MEFV* reverse sequence 5′-GAGGAGCTGTGTTCTTCCCTC-3′ and the result were analyzed by SnapGene software and online alignment using clustal Omega Multiple sequence Alignment.

Statistical analysis

We used the Statistical Package for the Social Sciences (SPSS 15) for statistical analyzing of our data. Also, we used a chi-square test, when appropriate, to compare the difference in the prevalence of exon 2 *MEFV* variants between refractory RA patients and healthy subjects. All P values were 2-tailed, and P values less than 0.05 were weighted significant. Descriptive findings were reported as mean ± SD.

Results:

The demographic features of study subjects are summarized in Table 1. Genotyping of exon 2 of *MEFV* gene failed for 4 refractory RA patients. Consequently, the statistical analysis was performed on the remaining 84 subjects with complete data for exon 2 of 34 refractory RA patients. The mean age of patients with refractory RA patients were 43.8 ± 7.7 years and disease duration was 49.8 ± 7.36 months. The mean of DAS 28 (Disease Activity score) of patients with refractory RA was 4.2 ± 0.51. The mean age of healthy control cases were 45.4 ± 8.27 years. There were 6 (17.6%) E148Q mutations in exon 2 of *MEFV* gene variations in refractory RA patients and 5 (10%) mutations in healthy subjects. The mutation frequency of exon 2 *MEFV* variants in the refractory subjects as compared with the healthy controls was similar (P=0.405). According to the result, we found no significant difference between patients with refractory RA and healthy subjects.

	Refractory RA patients	Healthy Subjects
Number	34	50
Median age (year), mean ± SD	43.8 ± 7.7	45.4 ± 8.27
BMI (kg/m2)	26.3	25.4
ESR	34.6	8.5
Women, No%	33 (97)	50 (100)

Table 1: Clinical and demographic description of refractory RA patients and healthy subjects.

Discussion

According to the result of this study, we found a high prevalence of E148Q mutation on exon 2 of *MEFV* gene in both groups, including patients with severe refractory RA and healthy subjects. However, the difference was not statistically significant. This result, indicating us that E148Q mutation is not positively associated with a predisposition to development of inflammatory arthritis including RA.

It is noteworthy that in preliminary studies, the result was the same [1,14,23,24]. Most studies which shown the positive effect of *MEFV* gene mutations on inflammatory arthritis performed on patients with ankylosing spondylitis (AS) [9,25,26]. Nevertheless, according to the results of these studies E148Q and other *MEFV* gene mutations may play role in severity of inflammatory arthritis [1,13,14]. To confirm this, we performed our study on patients with severe refractory RA and we expected a higher E148Q mutation rate in this patients group comparing with healthy subjects but the result of our study does not

confirm this and the overall E148Q mutation rate was similar in both groups.

In conclusion, the results of our present study, along with the published literature, indicate that the prevalence of exon 2 *MEFV* gene mutations is comparable between patients with refractory rheumatoid arthritis and healthy subjects and this mutation has no predisposing effect on RA and increasing disease severity. However, Because of small sample size this and other studies have low statistical power. Further studies with larger populations are required.

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