The Association Between Matrix Metalloproteinase-7 A-181G Polymorphism and the Risk of Relapsing-Remitting Multiple Sclerosis in Iranian Kurdish Patients from Kermanshah

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1. Background

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) that leads to progressive neurologic disorders. Multiple sclerosis is the most prevalent nerve disease among young adults (18 to 40 years old) that is accompanied by periods of relapse and remit. There are two million MS patients in the world (1). Studies demonstrate that the risk of this disease in individuals with a family history of MS is more than those without a family history, suggesting a genetic susceptibility to MS disease (2). This disease has four clinically different forms including relapsing-remitting multiple sclerosis (RR-MS), primary-progressive multiple sclerosis (PP-MS), secondary-progressive multiple sclerosis (SP-MS), and progressive-relapsing multiple sclerosis (PR-MS) (3).

The risk of RR-MS is twice in women compared to men. During this phase of the disease, the majority of patients with RR-MS will develop SP-MS that is the more intense form of the disease and is accompanied by worsening of the clinical symptoms and decrease in parenchymal volume of the brain (4).

The MMPs are a family of enzymes with different functions, which include 28 endopeptidases that have an important role in the degradation of basal membrane barriers. The target of MMPs enzymes is the extracellular matrix (ECM) including collagens, laminins, fibronectins and heparan sulfate proteoglycans, cell-cell adhesive molecules, cell surface receptors, growth factors, cytokines, chemotactic factors and other proteases (5). Increased MMPs activity might be one of the factors that cause progression of MS disease (5). Imbalance in the level of these enzymes has been detected in the serum and cerebrospinal fluid (CSF) of MS patients (6-8). These enzymes have a key role in the destruction of the blood-brain barrier (BBB) and central nervous system (7-9). Matrix metalloproteinase-7 (MMP-7) is an important member of MMPs family that af-
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In the present case-control study 80 RR-MS patients with the mean age of 35.9 ± 9.2 years, consisting of 65 females and 15 males, who had referred to the Neurology Department of Kermanshah University of Medical Sciences and also 80 healthy sex- and age- matched individuals with mean age of 34.8 ± 10.9 years, including 60 females and 20 males, without any symptoms of the disease were investigated. A complete neurological examination including expanded disability status scale (EDSS), which defines the degree of neurological impairment, was performed for the studied individuals. After obtaining an informed written consent from the studied individuals 5 ml of whole blood was collected from patients and normal controls. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences.

3. Materials and Methods

3.1. Sample

In the present case-control study, 80 RR-MS patients with the mean age of 35.9 ± 9.2 years, consisting of 65 females and 15 males, who had referred to the Neurology Department of Kermanshah University of Medical Sciences and also 80 healthy sex- and age- matched individuals with mean age of 34.8 ± 10.9 years, including 60 females and 20 males, without any symptoms of the disease were investigated. A complete neurological examination including expanded disability status scale (EDSS), which defines the degree of neurological impairment, was performed for the MS patients. After obtaining an informed written consent from the studied individuals, 5 ml of whole blood was collected from patients and normal controls. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences.

3.2. Genotype Analysis

Restriction enzyme of EcoRI was provided by the Roche Company (Germany). The 50-base pair (bp) molecular weight marker was supplied from the GeneOn Company (Germany). Polymerase chain reaction (PCR) reagents (Taq polymerase and dNTPs) were provided from the Sinaclon Company (Iran).

DNA extraction from peripheral blood was performed by phenol-chloroform method (14). The concentration of extracted DNA was calculated by the Nanodrop spectrophotometer system. A fragment with 150 bp promoter region of MMP-7 was amplified using the primer sequences (10) that are presented in Table 1. The accuracy of primers was confirmed by the BLAST program in NCBI data bank.

Polymerase chain reaction were performed in a 25 μl reaction mixture containing 300 ng of target DNA, 20 pmol of each primer, 1X PCR buffer, 1.5 mM MgCl2, 200 μM dNTPs, and 1 unit of Taq DNA polymerase. DNA was amplified by the following steps: an initial five minutes of denaturation at 95°C, followed by 35 cycles at 95°C for one minute, 62°C for one minute, 72°C for one minute, and 10 minutes of final elongation at 72°C. After amplification, 15 μl of PCR products were subjected to overnight digestion at 37°C with 2 units of EcoRI restriction enzyme and 2.5 μl of 10X buffer enzyme in a total volume of 25 μl. Digested PCR products were identified as AA homozygote (wild genotype) with 150 bp, GG homozygote (mutant genotype) with 120 bp and 30 bp, and AG heterozygote genotype with three fragments of 150, 120 and 30 bp. Digested products were visualized on a 3% agarose gel stained with Gel Red under ultraviolet light (10).

3.3. Statistics

The significance of the difference in alleles and genotypes frequencies between the patient and control groups was tested using the chi-square method. Odds ratios (OR) were calculated as estimates of relative risk for the disease and 95% confidence intervals were measured by logistic regression using the SPSS software. The results were considered statistically significant when P < 0.05. All of the statistical analyses were done using the SPSS statistical software package, version 16.0.

4. Results

Figure 1 visualizes the electrophoresis of amplified 150 bp fragment in the promoter region of MMP-7 on 1% agarose gel. Figure 2 shows the various genotypes of digested PCR products of MMP-7 polymorphism with EcoRI restriction enzyme.

The frequencies of MMP-7 AA, AG and GG genotypes in RR-MS patients were 25%, 47.5% and 27.5%, respectively compared with 32.5%, 55% and 12.5%, respectively in healthy controls (P = 0.057). There was no significant difference in the frequency of AG genotype in RR-MS patients and controls (P > 0.05). In RR-MS patients the frequency of MMP-7 GG genotype was significantly (P = 0.028) higher than that in controls (Table 2). The presence of GG genotype increased the risk of RR-MS by 1.69 times [OR = 1.69 and 95% CI = 1.05-2.72, P = 0.03].

As indicated in Table 2 the MMP-7 G allele was more prevalent in patients (51.2%, P = 0.043) compared to controls (40%). The presence of this allele increased the risk of RR-MS by 1.58 folds (P = 0.044).

Table 1. Primer Sequences used for Amplification of Matrix Metalloproteinase-7 Gene

<table>
<thead>
<tr>
<th>Primer Sequence</th>
<th>Location</th>
<th>Location</th>
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<tbody>
<tr>
<td>5’ TGGTACCAAGATGCCTGAATT 3’</td>
<td>Forward Primer</td>
<td>Promotor</td>
</tr>
<tr>
<td>5’ TCGTAAATGGCAAGGCAACATAATGTT 3’</td>
<td>Revers Primer</td>
<td>Promotor</td>
</tr>
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</table>

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5. Discussion

Multiple sclerosis is the most common cause of neurological disability in adults (15). The incidence and prevalence of MS especially among Iranian females is rapidly growing with the female to male ratio of 1.83:6, while in most parts of the country the ratio is more than 3. This high female to male ratio might be explained by the difference in lifestyle that is reflected in high prevalence of vitamin D deficiency among young Iranian women (16).

Matrix metalloproteinase (MMPs) are zinc-dependent enzymes that play a crucial role in restructuring the extracellular matrix by activating the secretion of gelatinases, collagenases and proteolytic enzymes (17, 18). Matrix metalloproteinase damages tissue in MS by two mechanisms. The first mechanism is the degradation of the endothelial lining of vessels that causes entry of inflammatory cells from the blood vessels into parenchyma. The second mechanism is activation of inactive forms of certain inflammation mediators by increased levels of MMPs. Breakdown of the myelin sheath within the CNS parenchyma by MMPs results in demyelination and inflammation within the CNS (19). The release of MMPs at the sites of inflammation and their role in degradation of the various components of the extracellular matrix (19, 20) indicates that the genetic variants of MMPs might affect the function of MMP enzymes.

The MMP-7-181G allele increases the MMP-7 gene transcription and promoter activity compared to the –181A allele. The higher expression of this allele is related to the presence of a putative binding site for a heat-shock transcription factor (21) resulting in increased protein expression. This binding site is absent in the A allele (22).

In the present study we observed that the MMP-7 GG genotype and also MMP-7 G allele increased the risk of RR-MS by 1.69 and 1.58 folds, respectively. It seems that higher promoter activity of the gene in the presence of this polymorphism, alterations in the gene expression or mRNA stability with consequences of increased protein expression results in higher degradation of ECM and non-ECM components that might affect the risk of RR-MS. However, this hypothesis remains to be confirmed.

There is no data available regarding the role of MMP-7 A-181G polymorphism in the pathogenesis of MS. However, there are a few studies that have reported the increased activity of MMP-7 in MS, experimental autoimmune encephalomyelitis and autoimmune neuritis (23-25).

In summary, the findings of the present study suggests that the GG genotype and G allele of the MMP-7 A-181G polymorphism are associated with the risk of RR-MS in the Kurdish population from Kermanshah and this polymorphism might be a susceptibility factor for RR-MS in our population.

Authors’ Contributions

Ziba Rahimi and Fatemeh Mohammadi performed the experiments. Zohreh Rahimi designed the study, analyzed and interpreted the data and wrote the manuscript.
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References