Inverse Relation between MxA Gene Expression and Age in Multiple Sclerosis Patients Reveals a Gender Difference in Response to Interferon Therapy

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ABSTRACT

Multiple sclerosis (MS) is an inflammatory, multifocal, immune-mediated disease of the central nervous system that women are at a higher risk to acquire than men. Myxovirus resistance protein A (MxA) is used as a predictive marker of bioactivity of interferon-beta (IFN-β) therapy in MS patients. This study was undertaken in west of Iran to investigate gender differences in the expression level of MxA in relapsing-remitting MS (RRMS) patients receiving IFN-β therapy, compared with untreated normal individuals.

The expression level of the MxA gene in RRMS samples were compared to untreated normal individuals using the extracted RNA from whole blood of 50 RRMS patients (31 females and 19 males) and 50 normal controls (29 females and 21 males). All patients were HLA-DRB1*15 negative and responded to IFN-β with a normal vitamin D level. The level of MxA gene expression was measured by quantitative RT-PCR.

The levels of gene expression were decreased in RRMS patients compared with normal counterparts (p=0.025). This decrease was significant in females (p=0.009) compared to males (p>0.05). The level of expression varied across different female age-groups with no significant difference in women younger than 30 years, but a significant decrease in expression in women between 30 to 40 years or above 40 years of age was seen.

There was neither linear correlation between the MxA expression level and risk of expanded disability status scale of Kurtzke (EDSS); nor were there any significant correlation between expression status of MxA and duration of the disease. In conclusion, the decrease in the level of MxA expression in MS patients treated with IFN-β when compared to normal individuals was significantly lower in females than males. This demonstrated a gender bias in the response to IFN-β therapy that will need to be confirmed and further investigated in more detail.

Keywords: Gene expression; Interferon-beta; Multiple sclerosis; Mx1

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INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease that can harm the central nervous system and result in mental and physical disabilities.\(^1\)\(^2\) This disease mainly affects middle-aged adults, but women may be 9 to 13 times at greater risk to acquire MS than men.\(^3\) There is no clear evidence as yet on immuno-pathogenic mechanisms involved in the development of the disease although, genetically predisposed people are more likely to contract MS.\(^4\)\(^6\) Environmental factors may intensify the disease and there are no known factors that suppress disease activity. Unfortunately, the number of people with MS is reportedly increasing in different countries,\(^7\) particularly in Iran.\(^8\) The inflammatory damages display the autoimmune trait of MS, in this regard association analysis and expression assessment for some of these susceptibility genes, especially HLA and cytokine genes, in Iranian patients were previously investigated.\(^9\)\(^11\)

The most common immune-modulatory therapy used for MS is interferon beta (IFN-\(\beta\)).\(^12\) However, the effectiveness of IFN-\(\beta\) therapy is difficult to monitor\(^13\) and the clinical response to IFN-\(\beta\) therapy may exhibit considerable variability in the number and types of MS relapsed cases.\(^14\) Moreover, the effectiveness of IFN-\(\beta\) therapy might vary between men and women because of the marked physiological differences between the genders in their immune response.\(^15\) For example, IFN treatment is sensitive to the regulation of the female hormone, estrogen.\(^16\) Estrogen affects all the major cells of the immune system including T and B cells, macrophages, dendritic cells and natural killer cells.\(^15\)\(^19\)

Recently, a number of studies have shown that the measurement of baseline levels of myxovirus resistance protein A (MxA) mRNA is a potential biomarker of IFN-\(\beta\) bioactivity,\(^20\)\(^22\) that can be used to predict the response of MS to IFN-\(\beta\) treatment.\(^23\)\(^25\) MxA is a 75-kDa cytoplasmic protein member of the dynamin superfamily that is induced by type I interferons (IFN-\(\alpha\) and IFN-\(\beta\)). MxA can inhibit the replication of single-strand RNA viruses, and is stable in the absence of viral infections.\(^25\) In addition; MxA has a possible role in the signal transduction of the apoptotic pathway.\(^26\)

Given the potential gender differences associated with MS, the aim of this study was to examine the differences in the gene expression levels of MxA in relapsing-remitting MS (RRMS) female and male patients receiving IFN-\(\beta\) therapy, compared with untreated normal individuals.

MATERIALS AND METHODS

Patients and Controls

Blood sample was collected from 50 RRMS patients (31 females and 19 males, mean age: 37.4±5.2, age of onset: 31.36±2.4, duration of disease: 6.1±3.1 years, expanded disability status scale EDSS: 2.72±2.3) during interferon treatment and also samples from 50 healthy controls (29 females and 21 males, mean age: 36.9±4.6) receiving no treatment. Clinical profiles of MS patients and healthy individuals are shown in Table 1. RRMS in patients was identified with magnetic resonance imaging (MRI), based on McDonald criteria.\(^27\)\(^28\) All of the patients were HLA-DRB1*15 negative,\(^29\) they were clinically stable, and received daily injections of IFN-\(\beta\) (CinnoVex, Cinagene Company, Iran) during period of blood collection.

Blood Sampling

A 5 mL peripheral blood sample was obtained from each participant–control and patients groups. The local Ethics Committee of Shahid Beheshti University of Medical Sciences approved (No. 3275) the collection of blood samples at Iran’s MS Society Clinic and Imam Hossein Hospital in Tehran.

Table 1. Demographic and clinical profiles of patients with relapsing-remitting multiple sclerosis and healthy controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>MS patient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/Male [no. (%)]</td>
<td>31 (62%)/19(38%)</td>
<td>29 (58%)/21(42%)</td>
</tr>
<tr>
<td>Age (mean ± SD, Years)</td>
<td>37.4 ± 5.2</td>
<td>36.9 ± 4.6</td>
</tr>
<tr>
<td>Age range (Years)</td>
<td>17-69</td>
<td>19-63</td>
</tr>
<tr>
<td>Age of onset (mean ± SD, Years)</td>
<td>31.36 ± 2.4</td>
<td>-</td>
</tr>
<tr>
<td>Duration (mean ± SD, Years)</td>
<td>6.1 ± 3.1</td>
<td>-</td>
</tr>
<tr>
<td>EDSS (mean ± SD)</td>
<td>2.72 ± 2.3</td>
<td>-</td>
</tr>
</tbody>
</table>

a: Expanded disability status scale of Kurtzke.
Table 2. The sequences of probes and primers for expression analysis of MX dynamin like GTPase 1 (MXA) gene and hypoxanthine phosphoribosyltransferase 1 (HPRT1) as reference gene

<table>
<thead>
<tr>
<th>Property</th>
<th>HPRT1</th>
<th>MXA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCBI accession number</td>
<td>BC000578</td>
<td>M30817</td>
</tr>
<tr>
<td>Forward primer</td>
<td>AGCCTAAGATGAGAGTTTC</td>
<td>CAGCACCTGATGCGCTATCAC</td>
</tr>
<tr>
<td>Amount of use</td>
<td>10 picomol</td>
<td>10 picomol</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>CACAGAACTAGACATTTGATA</td>
<td>GAGCATGAAGAACTGGATGATCAA</td>
</tr>
<tr>
<td>Amount of use</td>
<td>10 picomol</td>
<td>10 picomol</td>
</tr>
<tr>
<td>Probe</td>
<td>Fam-CATCTGGAGTCTATTGACATCGC-Tamra</td>
<td>Fam-AGCAAGCGCATCTCCAGCCACATC-Tamra</td>
</tr>
<tr>
<td>Amount of use</td>
<td>7.5 picomol</td>
<td>7.5 picomol</td>
</tr>
<tr>
<td>Amplicon length</td>
<td>101bp</td>
<td>81bp</td>
</tr>
<tr>
<td>Optimized annealing</td>
<td>60°C</td>
<td>60°C</td>
</tr>
</tbody>
</table>

Quantitative Real-Time-PCR
The RNA was extracted using Geneall Hybrid-R blood RNA extraction kit (cat No.305-101, Korea) according to the manufacturer's instruction. The cDNA was synthesized with Biosystems High-Capacity cDNA Reverse Transcription Kit (PN: 4375575, USA). Allele ID 7 (Premier Biosoft, Palo Alto, USA) was applied to design the sequence of the specific probes and primers shown in Table 2. The HPRT1 housekeeping gene was used as a positive control and internal reference in the expression studies. The Applied Biosystems TaqMan Universal PCR Master Mix (PN: 4304449, USA) was used to run the Real-time quantitative PCR in a Corbett Rotor Gene 6000 machine (Corbett Life Science, USA). The no template control (NTC) was used as a negative control in each run for each primer. The expression analysis for each sample was performed in triplicates.

Statistical Methods
Independent T-test was adopted in order to compare data obtained from participants. The one-way ANOVA test was also used to complete statistical computations. Mann–Whitney test was applied to test for differences in each group with small size, due to the skewed distributions of data. The p value for the level of significance was set at p≤0.05. The analyses were performed using the SPSS version 18 windows statistical package (Chicago, IL, USA).

RESULTS
MxA Expression Level and Risk of RRMS
The expression level of MxA gene in RRMS patients was compared with normal individuals (Table 3). The data for the total number of participants (i.e.,

Table 3. MxA expression level in relapsing-remitting multiple sclerosis patients, compared with control group, based on age and sex of the participants

<table>
<thead>
<tr>
<th>MxA expression</th>
<th>Control no.</th>
<th>RRMS patient no.</th>
<th>p value</th>
<th>Expression ratio</th>
<th>Std Error</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>50</td>
<td>50</td>
<td>0.025</td>
<td>0.478</td>
<td>0.498</td>
<td>0.123-2.536</td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>19</td>
<td>0.27</td>
<td>0.817</td>
<td>0.747</td>
<td>0.676-2.327</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
<td>31</td>
<td>0.009</td>
<td>0.358</td>
<td>0.501</td>
<td>0.455-2.447</td>
</tr>
<tr>
<td>&lt;30</td>
<td>Male</td>
<td>9</td>
<td>5</td>
<td>0.75</td>
<td>0.91</td>
<td>1.813</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>13</td>
<td>11</td>
<td>0.11</td>
<td>0.612</td>
<td>0.663</td>
</tr>
<tr>
<td>30-40</td>
<td>Male</td>
<td>7</td>
<td>6</td>
<td>0.71</td>
<td>0.8</td>
<td>1.615</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9</td>
<td>9</td>
<td>0.03</td>
<td>0.301</td>
<td>1.144</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Male</td>
<td>5</td>
<td>8</td>
<td>0.24</td>
<td>0.69</td>
<td>1.174</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>7</td>
<td>11</td>
<td>0.01</td>
<td>0.212</td>
<td>0.792</td>
</tr>
</tbody>
</table>

RRMS, relapsing-remitting multiple sclerosis;
age and sex combined) revealed a statistically significant decrease in MS patients, compared to normal controls ($p=0.025$, expression ratio=0.478, 95% CI=0.123-2.536).

Comparing sex-linked subgroups of patients with normal individuals, the female patients showed a statistically significant decrease ($p$ value=0.009, expression ratio = 0.358, 95% CI=0.455-2.447), whereas the male patients only showed a slight non-significant decrease in MxA expression ($p=0.27$).

The level of expression varied across different female age-groups, with no significant difference in women younger than 30 years ($p=0.11$), but a significant decrease in expression in women between 30 to 40 years ($p=0.03$) or above 40 years of age ($p=0.01$).

The Taqman Q-RT-PCR results are demonstrated as fold changes in the graphs (Figure 1).

**MxA mRNA Expression Level and EDSS**

The correlation between EDSS and MxA relative quantitation was measured in the RRMS patients group and no significant linear correlation ($R^2=0.1876$) was found between EDSS and MxA expression (Figure 2).

![Figure 1. Fold change of MxA gene expression by Real time-PCR comparing multiple sclerosis patients of different ages](image1)

![Figure 2. Correlation between MX dynamin like GTPase 1 MxA relative quantitation and disease severity (expanded disability status scale, EDSS scores) in multiple sclerosis patients](image2)
MxA Gene Expression in MS

MxA Expression and Disease Duration

The correlation between MxA relative quantitation and disease duration in RR-MS patient group is shown in Figure 3. No significant correlation ($R^2=0.0181$) was found between expression status of MxA and duration of the disease.

DISCUSSION

MS is known as an immune-mediated demyelinating disease of the central nervous system, which is treated mostly by IFN-β. However, some patients do not respond to this treatment as effectively as expected. Since IFN induces MxA proteins in brain tissue of MS patients, the MxA mRNA level has been used to predict relapses in patients diagnosed with MS. Single-nucleotide polymorphism (SNPs) on the MxA promoter region appear to have an important role in the pathophysiology of MS patients, and increased levels of MxA and IFN in MS patients not treated with IFN have been attributed to an increase in viral infections. Thus, the reliability of MxA as a biological marker for monitoring IFN-β therapy has been questioned in a number of studies.

In this study we compared the expression levels of the MxA gene in RRMS patients with those of normal controls. The results showed a significant association between the age and sex of the patients, but not with the severity of their disease. That is, the MxA levels significantly decreased in women with RRMS, who were over 30 years of age. The male RRMS patients; however, did not show a similar pattern and there was no statistically significant decrease in their level of MxA. In contrast to the women, the male MxA expression level did not fluctuate across different age groups. These results are consistent with the known gender differences for MS as women are 9 to 11 times more likely to acquire MS than men. The female and male sex hormones are known to enhance IFN action. For example, estrogen was found to double the effect of IFN-γ and also to have a smaller additive effect for IFN-α in mouse cells. Therefore, it is likely that the decrease in the MxA expression levels in women over 30 years of age was due to a reduced effect of IFN therapy in menopausal women. This increase in IFN resistance during menopause may result directly or indirectly from the drop in the production of female sex hormones or from changes associated in the production and regulation of cytokines and other host factors. In addition, the sex differences is reported to make women more susceptible for developing autoimmune diseases than men because of the role of sex chromosome complement in female bias and the increased cellular and hormonal immune responses in women. Therefore, it should be considered to explain the observed gender differences for MS in this study and for future analyses of IFN resistance. Moreover, more studies are needed to elucidate the role of sex hormones in the regulation of MxA expression in MS patients.
of sex hormones and other gender factors that might affect IFN treatment for MS or other autoimmune diseases or viral infections. However, the important question still remains that if IFN induces MxA, then why did MxA decrease in responder patients who were taking IFN? The answer could be that MS patients without IFN injections had significantly lower levels of MxA expression compared with those who had been treated with IFN. Therefore, further studies are needed not only on patients being treated with IFN but also on patients who are not taking the same medications. Another question that will need to be resolved is whether sex hormone therapy in conjunction with IFN treatment in menopausal women might help restore the effectiveness of IFN treatment and raise the MxA gene levels. All together, the present study revealed that the decrease in the levels of MxA expression in MS patients treated with IFN-β when compared to untreated normal individuals was significantly lower in females than males. This demonstrated a likely gender bias in the response to IFN-β therapy.

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MxA Gene Expression in MS