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# MICB Gene Expression on Peripheral Blood Mononuclear Cells and Susceptibility to Multiple Sclerosis in North of Iran

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## ABSTRACT

Multiple sclerosis (MS) is an autoimmune multifactorial degenerative disease with detrimental affliction on central nervous system. MHC class I chain- related geneA,B(MICA and MICB) are nonclassical human leukocyte antigens that can affect on some diseases and also on transplantation.

The purpose of this study was to evaluate the MICA and MICB MRNA expression in multiple sclerosis patients. In this study, we evaluated MICA and MICB MRNA expression in the pripheral blood mononuclear cells by reverse transcryptase-polymerase chain reaction(RT-PCR) in MS patients and normal controls.

The results of this study showed that 32.6% of patients with progressive clinical outcome over expressed MICB genes in comparison with controls ($p$=0.002).

It is concluded that the high expression of MICB gene in MS patients is an important criterion of MS disease that it may be due to the interaction between MICB and its receptor on $CD8^+$T or NK cells.

**Keywords:** MICA; MICB; Multiple sclerosis

## INTRODUCTION

Multiple sclerosis (MS) is an autoimmune inflammatory disease with demyeliniation of the neurons in the nervous system.[1] It has been shown that genetical and enviromental factors can be associated with this disease.[2]

It is well established that Major histocompatibility complex (MHC) is among the molecules that has a key effective role on genetic susceptibility in MS patients.[3,4] Recently, it has been suggested that MHC class I chain- related gene A, B (MICA, B), which are located close to HLA-B locus, are important polymorphic genes in the genetic susceptibility to autoimmune diseases.[5] The levels of these antigens on

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the cells increase after stress and infections.[6] In addition, it has been shown that these glycoproteins express on the cancer cells.[7] These molecules are recognized by activating NKG2D receptor which is constitutively expressed on the natural killer cell, CD8+T cell and γδ Tcell.[8] The structure of MICA,B protein is similiar to classical MHC-I without beta2 microglubolin on the cell surface**.** Some studies demonstrated association of MICA, B genes with some diseases such as diabetes and behçjet disease.[9,10] The presence of MIC protein on the peripheral blood mononuclear cells impart an important out come in the autoimmune diseases pathogenesis.[10] While the association of MICB,A with multiple scelerosis is not clear, in this study, we evaluated expression of MICA,B in periphearl blood mononuclear cells in MS patients in comparison with normal control.

## MATERIALS AND METHODS

### Patients and Samples

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinzed blood by centrifugation on a Ficoll histipaque 1.077 (lymphoprep, Norway) which was donated to 48 MS patients with mean age ± SD, 36.08 ±7.97 and 48 normal control with age 30.23± 4.16 (Table1). Cells in interphases were collected and washed three times with RPMI-1640 medium (sigma, USA ). The number of cells were counted and then, the viability of cells was determined by trypan blue exclusion.

The diagnosis of multiple sclerosis was made according to the MacDonald criteria. The patients who took part in this study were informed clearly of the nature of this research and they gave inform consent for taking blood sample. The study has been approved by ethical board of Mazandaran university of medical sciences.

### mRNA Extraction ,cDNA and RT –PCR

Total mRNA was extracted from 5× $10^6$ pripheral mononuclear cells of MS patients and controls using RNA extraction kit (Qiagen, USA) according to manufacture instruction. Then, extracted mRNA was checked for its quality with electrophoresis in 1.5% agarose gel. The transcriptional levels of MICA,B of MS patients evaluated by reverse transcriptase PCR in comparison with control. The cDNA was synthesized using 2 µg of total RNA in 20 µl reaction mixture, consisting of 4 µl 5X reaction buffer: a combination of 10mM dNTPs 2 µl; 100 µM dithiotheritol (DTT) 1.5 µl; 10pMol/ml random hexamer(N6) 1 µl, and M-MLV reverse transcryptase 200units(fermentase, Italy). Thereafter, the mixture was incubated at 42ºC for 45 min and followed in 72 ºC for 5 min.

**Table1. Demographic data of multiple sclerosis patients and normal controls who participitated in the study**

| Clinical data | Patients (No=48) | Normal control (No=48) |
|---|---|---|
| Age(Mean ±SD) | 36.08 ± 7.97 | 30.23± 4.16 |
| Sex(M/F) | 18/30 | 19/29 |
| Drug | Avonex, Cinorex, rebif | - |
| Disease duration(year) (Mean±SD) | 6.28± 4.68 | - |

**Table2. Primer specifications for RT-PCR**

| Gene | Primer | sequences(5-3) | bp |
|---|---|---|---|
| MICB | Forward | ACACCCAGCAGTGGGGGGAT | 442 |
| | Reverse | AGCAGTCGTGAGTTTGCCCAC | |
| MICA | Forward | ACACCCAGCAGTGGGGGGAT | 677 |
| | Reverse | GCAGGGAATTGAATCCCAGCT | |
| B-Actin | Forward | TGGCCACGGCTGCTTCCAGC | 321 |
| | Reverse | AGGAGGAGCAATGATCTTGAT | |

**Specific PCR for MICA, Band Beta Actin**

After cDNA synthesize, the specific PCR was carried out by 10pmol primers for amplification of MICA,B genes. The PCR product was analazed by electrophoresis on 1% gel agarose. In addition, we used beta actin primers as internal control in this research (Table 2).

Briefly, 25µl reaction mixture of PCR was prepared using 2.5 µl of 10x buffer , 2 µl of 25mM mgcl2, 0.5 µl dNTPs(10Mm), 1 µl of each primer(10pmol) , 1 µl cDNA and 1 unit of Tag DNA polymerase (fermentase, Italy ). In PCR protocol we inculded 30 cycles, initiated by 1 cycle at 94ºc for activating the Tag DNA polymerase followed by 94º C ; 20sec , 63ºC ; 50sec , 72º C; 30sec and final extension for 10min at 72º C. The size of PCR product for MICA ,MICB and beta actin were 442bp ,677bp and 321bp.

**Statistical Analysis**

Data are presented as mean ± SD. For statistical analysis, we used paired *t*-tests.The *P* values (<0.05)were determined in all cases.

## RESULTS

**MICA,B MRNA Expression on PBMC by IFN-β**

Peripheral blood mononuclear cells from MS patients and controls were analyzed for expression of MICA,B and beta actins genes by RT-PCR(Figure1). After electrophoresis on agarose, we measured density of PCR product bands by image J software (Table 3).

**cDNA Synthesis by Reverse Transcryptase**

In 15 patients, MICB overexpressed on PBMC in comparison with controls( *P*= 0.002). The results are presented in table 3.

**The Clinical Outcome of MS Patients**

MS patients with MICB over expression showed a serious clinical outcome in comparison with normal control. The clinical symptoms of two groups of MS patients are presented in table 4.

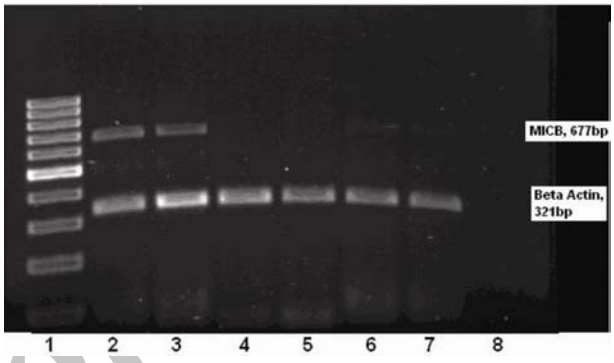


**Figure1. RT-PCR analysis of MICB and beta actin (as a house keeping gene) expression in Multiple sclerosis patients and controls after treatment with IFN-β. The cDNA samples consisted of DNA weight marker 8 (lane1); MICB expression from MS patients (line 2,3,6,7); cDNA from MS patients without MICB expression(4,5); and negative control ( line8).**

**Table3. MICB MRNA expression in MS patients in comparison with normal controls that were determined by image J software. The results were quantified by PCR products band density(Mean ±SD).**

| Sample | Gene expression | *p* |
|---|---|---|
| Ms patients | 115.70±34.13 | |
| Healthy control | 65.4± 3.77 | 0.02 |

**Table4. Comparison of Clinical data of MS patients with normal control**

| Disorders | MS patients with over expression of MICB (32.6%) | MS patients with low expression of MICB( 67.4%) | Healthy control |
|---|---|---|---|
| EDSS Score | 1.5 ± 1.2 | 1.2 ± 0.7 | - |
| Visual | Progressive | Non progressive | - |
| Sense | Progressive | Non progressive | - |

EDSS= Expanded Disability Status Scale

## DISCUSSION

In the present study, we evaluated MHC class I chain- related gene expression in the peripheral blood mononuclear cells of MS patients in comparison with control. There are studies which demonstrated that cell bearing MHC class I chain- related gene acquires potential to regulate various immune activities in cancer.[11,12] Our results showed that MICB MRNA upregulated in 32.6% of MS patients in comparison to normal controls. It has been shown that dysregulation of MHC class I chain- related gene receptors, NKG2D, can lead to autoimmune diseases by activation of autoreactive T cells.[13-15] In this study, MICA gene rarely expressed on healthy controls and MS patients cells. Thus, MICA may not be associated with unwanted immune responses in multiple sclerosis.

MICB, as a ligand for NKG2D, can stimulate immune cells against transformed cells, therefore, MICB expression can lead to higher capacity of activation of T cells through NKG2D receptor , leading to autoreactive T cell stimulation. Interaction of MICB with its recetor on the NK or $CD8^+$ T cells results to target cytolytic activity.[16,17] Morera et al. showed that soluble forms of MIC molecules in sera of MS patients led to progression of disease.[18]

Jinushi et al. demonstrated that MICA,B in hepatocellular carcinoma has an effective role in the cancer cell cytolysis by NK cells.[19] In this study, MS patients showed expression of MICB molecule on mononuclear cells in particular in those who showed progressive symptomes of disease with serious clinical outcome. Thus, over expression of MICB genes related in patients with severe disease and its expression may be associated with degeneration of myelin in central nervous system. It is concluded that high expression of MICB gene in MS patients can be one of key factors in autoimmune diseases because of reaction with NKG2D on $\alpha\beta CD8^+$T cells, $\gamma\delta$ T cells or NK cells.

Accorting to our data, we would like to suggest that low or no expression of MICB has a beneficial effect to prevent of autoimmune response in the MS patients.

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