

Analysis of Micro-RNA-144 Expression Profile in Patients with Multiple Sclerosis in Comparison with Healthy Individuals

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Abstract

Background: Etiology of multiple sclerosis is non-clarified. It seems that environmental factors impact epigenetic in this disease. Micro-RNAs (MIR) as epigenetic factors are one of the most important factors in non-genetically neurodegenerative diseases. It has been found MIR-144 plays a main role in the regulation of many processes in the central nervous system. Here, we aimed to investigation of MIR-144 expression alteration in Multiple sclerosis (MS) patients.

Methods: In this study 32 healthy and 32 MS patient's blood sample were analyzed by quantitative Real-Time PCR method and obtained data analyzed by REST 2009 software.

Results: Analysis of Real-Time PCR data revealed that miR-144 Increase significantly in MS patients compared to healthy controls.

Conclusions: The increase of MIR-144 expression in MS patients is obvious. MIR-144 can be used as a biomarker of MS and help to early diagnosis and treatment of this disease.

Keywords: MicroRNA (miRNA), MiRNA-144, Multiple Sclerosis (MS).

Introduction

Multiple sclerosis (MS) is an inflammatory disorder with a multifactorial etiology, which is associated by the disruption of myelin in axons of the central nervous system (CNS). In MS patient's CNS, immune system attack to myelin sheath and disrupt the axons of nerve cells, which is the principal purpose of this disorder (1, 2). This changes finally result in inflammation (3), active and inactive lesions (4, 5), remyelinated shadow plaques (6), and the myelination in the gray matter (7), which is most noticeable in the progression of the disease. Although this chronic disease is rare

in tropical countries except for Israel and Palestine, its endemic in peoples apart of equator orbits such as Indians of Canada and America, and New Zealand's people (8, 9).

Although MHC genes, like HLA-class 2 complex, HLA-DR15Dw2, and DQW6, are candidates of MS association, there is evidence that it is not a single-gene disorder and associated by the variety of environmental factors, which is the main reason for classifying of MS as a sporadic disease (10, 11). These suggest that, although genetics may play a role in this disease, the effects of

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environmental and epigenetic factors are more important (12). Sunlight and vitamin D are environmental factors, which thought to be involved in some cases of MS (13, 14). Also, detection of some viruses' genome such as Epstein-Barr virus (EBV), and human herpesvirus-6 (HHV6), were reported in many cases of MS (15, 16). Most of the environmental factors involved in the disease affect the expression of IL-10 and an increase in this cytokine causes the immune system to respond. Eventually, this autoimmune attack induces inflammation and the development of this neurodegenerative disease (17, 18). Epigenetic mechanisms include post-transcriptional changes of histones, DNA methylation, and expression of miRNAs. Meanwhile, miRNAs are involved in inherent and acquired immunity through the development and operation of granulocytes, monocytes, macrophages, dendritic cells, natural killer cells, and differentiation of B cell and T cells (19, 20). Also, miRNAs are involved in autoimmune diseases and central nervous system (21, 22). miRNAs can cause degeneration of neurons, loss of axons and mitochondrial function impairment in MS patients (20, 23). For example, next-generation sequencing and microRNA array analysis of the blood of patients with MS and healthy controls revealed that miR-16-2-3p was over-expressed in patients, whereas miR-7-1-3p and miR-20a-5p were down-regulated (24). Moreover, a new Real-time quantitative PCR analysis showed that miR-142-3p, miR-146a, miR-155, and miR-326 were up-regulated in MS patients (25). An increase in the relationship between different miRNAs with MS causes the need for increased studies in this regard.

The MIR-144 family is located on chromosome 17 and includes mir4732, mir451b, mir-451a, mir144 (26). According to previous studies, miRNA-144 plays an important role in myelination (27). Due to myelin sheath damages in MS disease, it seems that MIR-144 is a suitable candidate for more studies.

In this study, we aimed to investigate the effect of 144Micro RNA expression on the Iranian population.

Materials and Methods

Samples

In this study 32 healthy and 32 MS patient's blood sample were analyzed. The number of samples calculated based on Cochran formula

$$n = \frac{z^2 pq}{d^2 \left(1 + \frac{1}{N} \left[\frac{z^2 pq}{d^2} - 1 \right] \right)}$$
 in this formula The: **n** is the size of the sample, **N** is the population size, **p** is the proportion of population, **q**= 1-p, **z**= 1.96 and **d** is the margin of error (0.05). The mean age of patients was 22-48 years. The patients were new cases by the mean duration of the disease in 3 years, and also did not take any immunosuppressant drug. Healthy people and patients with MS were approved by a neurologist. Then, three ml of venous blood was taken from each subject in EDTA blood tube and transferred to the laboratory.

RNA Extraction and cDNA synthesis

RNX-Plus kit (Sinaclon, Iran) was used for extraction of blood sample's RNA according to kit's protocol. So extracted RNAs were loaded in the electrophoresis gel due to RNA's qualitative measurement. NanoDrop was used to measurement of RNA concentration and A260nm/A280nm ratio confirmed the purity of RNA. cDNA from total RNA of samples were synthesized by M-MuLV kit (Sinaclon, Iran) based on protocol.

Real-Time PCR

MIR-144 primer sets designed by oligo7 software and blasted in NCBI. Primers are listed in Table-1. Primers qualified by gel electrophoresis and used to quantitative SYBR-Green Real-Time PCR, which carried out using the master mix by Applied Biosystem StepOne instrument. The Real-Time PCR raw data was gathered in StepOne's Applied Biosystem software. By measuring the fluorescence readmitted by the device, the important parameters such as Cycle threshold, Ct, ΔCT, ΔΔCT, and Efficiency are calculated for reference gene and the mir-144 in both healthy and patient groups. Then data was analyzed by REST 2009 software.

Data analysis

Raw data of Real-Time PCR obtained from Applied Biosystem StepOne software and important factors such as cycle threshold (Ct),

Δ ct and $\Delta\Delta$ ct calculated for all healthy and patient samples based on a previous study (28). Then the collected data was analyzed using REST 2009 software.

Table 1. sequences of primers.

Primers	Sequences of primers	primer's length
miR- 144	Forward: 5`GCCTACAGTATAGATGATGTAC 3`	21
	Reverse: 5`GCGAGCACAGAATTAATACG 3`	20
5SrRNA	Forward: 5` CGGCCATACCACCTGAAC 3`	18
	Reverse: 5` CCTACAGCACCCGGTATTC 3`	19

Results**Results of quantitative PCR**

Fold change was calculated based on the ratio of miR-144 expression to 5SrRN. $\Delta\Delta$ ct calculated and then analyzed according to the Livak method. The Fold-Change value was 0.379604346 in our study. The results of MIR-144 expression in healthy and patients with multiple sclerosis are shown in Table-2. These results indicate that MIR-144 expression has significantly decreased in patients with multiple sclerosis ($p \leq 0.05$).

Results of statistical analysis by Rest-2009

After performing Real-time PCR and obtaining CT values. We used Rest software to analyze the $2^{-\Delta\Delta$ ct. The results indicates that the miR-144 expression increases in the patients compared with the healthy subjects ($p \leq 0.05$). The results of Rest-2009 software are presented in Fig. 1.

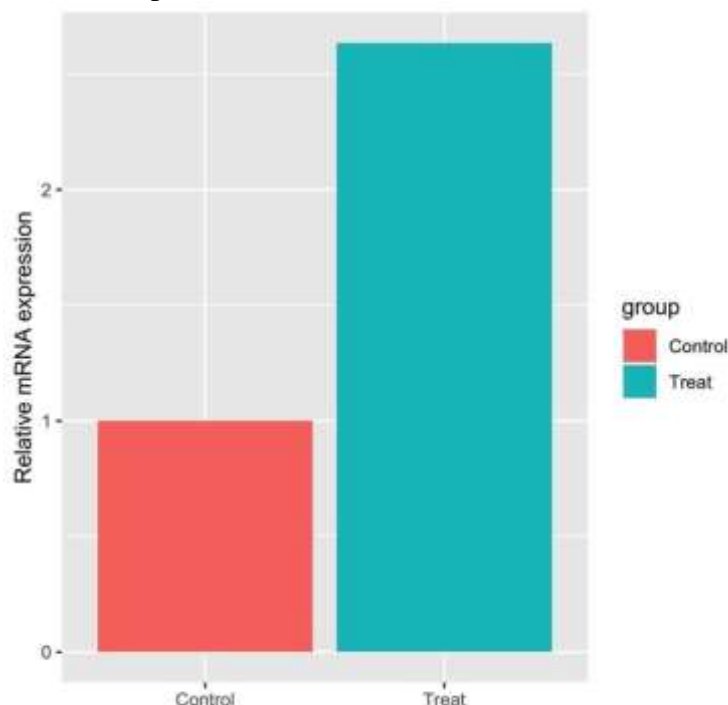


Fig. 1. The expression of miR144 in the statistical population studied in the Rest 2009 software.

Discussion

Multiple sclerosis is an acute autoimmune inflammatory disease which impacts on the central nervous system. MS patients abnormally have a large number of T lymphocyte helpers in their cerebrospinal fluid (29). There is still no definitive cause and treatment for the disease. It seems that a combination of genetic and environmental factors are involved in the development and progression of the disease. Biomarkers at an early stage can prevent the disease progression (30). Recently, micro-RNAs have appeared as key regulators of the differentiation of the immune cell line, maturity, maintenance of homeostasis, and normal immune function. They are small and non-coding ribonucleic acids that play a key role in regulating the expression of the host genome at the post-transcriptional level. Slight changes in the expression of a microRNA lead to significant changes in the expression of different genes (31). Hence many micro-RNAs have been studied in MS patients. For example, based on research conducted in 2009, Du et al. showed that miR-326 plays an important role in MS pathogens by increasing the differentiation of damaged Th-17 cells (32). Sievers et, al. showed that expression of MIR-19b-2 decreases in MS patients compared by healthy controls (33). On the other hand, MIR-155 plays a significant role in the immune system of mammals, especially in the regulation and differentiation of T-cells (34, 35). MiR-144 has not yet been studied in MS but studied in various other diseases. Persengiev et, al. based

on Genome-wide analysis of miRNA expression revealed that MiR-144 regulates the expression of ataxin 1. Ataxin 1 gene is necessary for the development of spinocerebellar ataxia type 1 (SCA1). MiR-144 activity increased in the cerebellum and cortex of SCA1 and Alzheimer patients in comparison of healthy aged brains (36). Current studies have demonstrated that miR-144 is involved in tumor development and cardio-cerebrovascular disease (37). In recent years, it has become apparent that miRNAs have a role in gene expression regulation. These molecules can be effective in different fields such as early detection, disease risk assessment, monitoring progress, therapeutic responses to the drug, and ultimately treatment. So in this study, we aimed at the investigation of MIR-144 expression alteration in MS patient. In this regard, Real-Time PCR has been used to quantitation of MIR-144 expression in MS patient and healthy groups. This study demonstrated that MIR-144 expression decreased in MS patients in comparison of healthy controls, and this suggests that MIR-144 can be used as a biomarker of MS disease. Furthermore, MIR-144 is as a suitable therapeutic target for MS patients.

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