Distinctive deregulation of miR-27a and miR-27b in relapsing remitting multiple sclerosis

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Abstract

Introduction: Previous studies have proposed that microRNAs (miRNAs) expression might be responsible for immunological features associated with multiple sclerosis (MS) pathogenesis. We aimed to elucidate the alternation in *miR-27a* and *miR-27b* expression in relapsing-remitting multiple sclerosis (RRMS) patients compared to the healthy subjects.
Materials and methods: In this study, the expression levels of *miR-27a* and *miR-27b* were evaluated in peripheral blood samples of 60 RRMS patients (30 recurrence patients and 30 patients two months after relapse) and 30 healthy subjects by quantitative real-time PCR.
Results: The findings indicated that the expression of *miR-27a* was significantly decreased in recurring patients ($P<0.0001$) and two months after relapse patients ($P<0.003$) in comparison with healthy subjects. Moreover, *miR-27b* showed down regulation in recurring patients ($P<0.001$) and two months after relapse patients ($P<0.002$).
Conclusion: The results demonstrated an association between expression of miRNAs studied and RRMS disease during recurrence and two months after relapse. However, further research is warranted to confirm observed associations.

Keywords: *microRNA*, *miR-27a*, *miR-27b*, Multiple sclerosis

Introduction

Multiple sclerosis (MS) is an autoimmune disease caused by chronic inflammation of central nervous system (CNS) that gives rise to focal lesions in the white matter of the brain and spinal cord (1). Previous observations have demonstrated that MS disease has four classes including relapsing-remitting(RR), secondary progressive (SP), primary progressive (PP), and progressive relapsing (PR) (2-3). According to investigations, up to two million people worldwide got involved with MS. Clinical advent start between the third and fourth decade of life. There is also a higher prevalence among women than men and one out of every three women affected (4). Biomarkers could predict the development of MS in high-risk populations

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and allow for intermediacy strategies that may prevent evolution to definite disease (5). MicroRNAs (miRNAs) known as short non-coding RNA molecules 19–25 nucleotides long, leading the control of different biological processes. Regulation gene expression post-transcriptionally by binding to the 3′-untranslated region (UTR) or 5′-UTR of their mRNA targets (6). Previous studies have shown that abnormal miRNAs function in peripheral blood immune cells as well as CNS glial cells (7). In MS disease, miRNAs dysregulation is reported in several immune cells. Various investigations have indicated changes in miRNAs expression in brain tissue and immune cells from MS patients, and associations between MS progression and miRNAs expression. The main issue in MS is to expand biomarkers that could help in diagnosing MS disease (8-10). The up-regulation of *miR-27b*, *miR-128* and *miR-340* have been observed in $CD4^+$ T cells from patients with MS (11). Additionally, 13 miRNAs, including *miR-27a-5p, miR-29a-3p, miR-29b-1-5p, miR-29c-3p*, *miR-95*, *miR-149-5p*, *miR-181c-3p*, *miR-193a-3p, miR-193-5p, miR-423-5p*, *miR-532-5p, miR-708-5p* and *miR-874* were decreased in peripheral blood from MS patients after IFN-β therapy (12). In vitro treatment of oligonucleotide miRNA inhibitors for *miR-27b*, *miR-128* and *miR-340* induced the restoration of Th2 responses in T cells from MS patients. The results suggest that in vivo silencing of *miR-27b*, *miR-128* and *miR-340* may potentially be used in the treatment of MS patients (13). Few studies reflecting the exact role of miRNAs in generation or progression of MS are available. Therefore, we focused on miRNAs, which have been predicted to have important roles in the pathogenesis of MS. This study aimed to investigate the expression patterns of *miR-27a* and *miR-27b* in peripheral blood samples of MS patients.

Material and methods

Totally, 90 blood samples were collected from 60 relapsing-remitting multiple sclerosis (RRMS) patients, including 30 patients during recurrence and 30 patients two months after relapse and 30 healthy subjects that randomly selected at Kashani Hospital (Isfahan, Iran). The healthy individuals had no records of autoimmune disease, based on medical checkup. The RRMS patients were diagnosed by an expert neurologist based on the recommended McDonald diagnostic criteria (14). Four ml of peripheral blood was collected into EDTA-containing tubes and transported to the laboratory on ice.

PBMC isolation and miRNA extraction

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples by density gradient lymphoprep (Bio Sera, Kansas City, USA) based on the manufacturer's protocol. Briefly, 4 ml of blood was diluted with 4 ml physiological saline and gradually added to the 4 ml of lymphoprep gradient solution in a falcon tube. Collected falcons were centrifuged at 800 g for 30 min and then PBMCs were transferred from the middle phase into a 2 mL RNAase-free microtube and was frozen. miRNA was extracted from PBMCs using miRNA Hybrid-R (Geneall, Seoul, Korea) based on the manufacturer's instructions. The quality of miRNA at a 260/280 nm wavelength ratio was measured by a NanoDrop spectrometer (Thermo Scientific, Waltham, MA, USA).

cDNA synthesis and quantitative real-time PCR

cDNA synthesis for miRNA transcripts was performed using a universal cDNA synthesis kit (Exiqon, Denmark) according to the manufacturer's instructions and poly-A tailing method. In order to obtain suitable

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miRNAs associated with MS disease, comprehensive search in the miRWalk 2.0 database was performed. The real-time quantitative PCR reactions were accomplished in duplicate by using a Rotor-Gene 6000 (Corbett Life Science, Mortlake, Australia). 1 of each cDNA product was added to the master mix, contained 0.5 µl of each forward and reverse primers (Exiqon, Denmark), 5 µL of SYBR premix ExTaq II (TaKaRa, Kusatsu, Shiga Prefecture, Japan) and 3 µL of diethylpyrocarbonate-treated (DEPC) water. Moreover, U6 was quantified as the reference to normalize differences in total miRNA levels. The PCR conditions were as follows: 95 °C for 15 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. Data analysis was accomplished using the $2^{-\Delta\Delta CT}$ method (15).

Statistical analysis

Graph Pad Prism statistical version 5.01 (Graph Pad, San Diego, CA, USA) was applied for statistical analysis. The normality of the data was evaluated using the Kolmogorov–Smirnov test and one-way ANOVA was used to analyze the data. For all tests, $P<0.05$ was considered as the significance threshold.

Results

In this investigation, 60 RRMS patients were participated of whom 30 were recurrent and 30 were two months after relapse and 30 healthy subjects selected for the study as controls. Patients and controls were sufficiently matched in terms of age and sex. Biological features of two groups of patients (recurrent and recurrences for at least two months) and healthy subjects are shown in Table 1.

The expression of *miR-27a* and *miR-27b* was evaluated by quantitative real-time PCR method in two groups: RRMS patients (recurrence and two months after relapse) healthy subjects. The Ct values of real-time PCR were determined by $2^{-\Delta\Delta Ct}$ method. The relative expression level of *miR-27a* ($P<0.0001$ and 0.003, respectively) and *miR-27b* ($P<0.001$ and 0.002, respectively) was found to be significantly reduced in both RRMS patients compared to healthy subjects. The results showed that *miR-27a* and *miR-27b* was significantly decreased in the patients, thus, probably these miRNAs play important role in MS disease (Figure 1).

Discussion

One of the most common reason of neurological disability among young individuals is MS which has clinical course varies greatly. Various mechanisms of axonal damage-neurodegeneration, gliosis, and remyelination-repair combine together in different degrees to make a unique clinical result for patients. Therefore, guiding investigations to distinguishing dependable biomarkers for each independent MS pathogenic factor is of primary importance (16). miRNAs dysregulation has been proposed in several immune cells of MS patients; some investigations have indicated alternations in the expression of miRNA in the brain tissue and immune cells of MS patients and associations between MS progression and miRNA expression (17, 18). This investigation was performed to examine the expression of *miR-27a* and *miR-27b* by reverse transcriptase quantitative real-time PCR technique. The current study is the first study on the association of selected miRNAs expression in RRMS using RT-qPCR in an Iranian population.

Numerous studies showed the correlation between *miR-27a* and *miR-27b* with various autoimmune diseases like MS and T cells. In an investigation, 26 miRNAs containing *miR-15a, miR-15b, miR-16, miR-17, miR-20a, miR-20b, miR-27a, miR-27b, miR-93, miR-98, miR-106a, miR-126, miR-126, miR-140-5p, miR-211, miR-374a, miR-454, miR-*

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510, miR-579, miR-623, miR-624, let-7d, let-7f, let-7g and *let-7i* remarkably down-regulated in peripheral blood of patients with all MS subtypes (19).

Table 1. Biological features of recurring and two months after relapse patients and controls.

Samples	Age (year)	Age range	Drug used	Male (n)	Female (n)
Recurring patients	39.20 ± 2.154	18-60	Interferon: 18 Non-interferon: 12	7	23
Two months after relapse patients	33.7 ± 1.522	21-45	Interferon: 11 Non-interferon: 19	9	21
Controls	38.60 ± 1.843	21-58	-	10	20

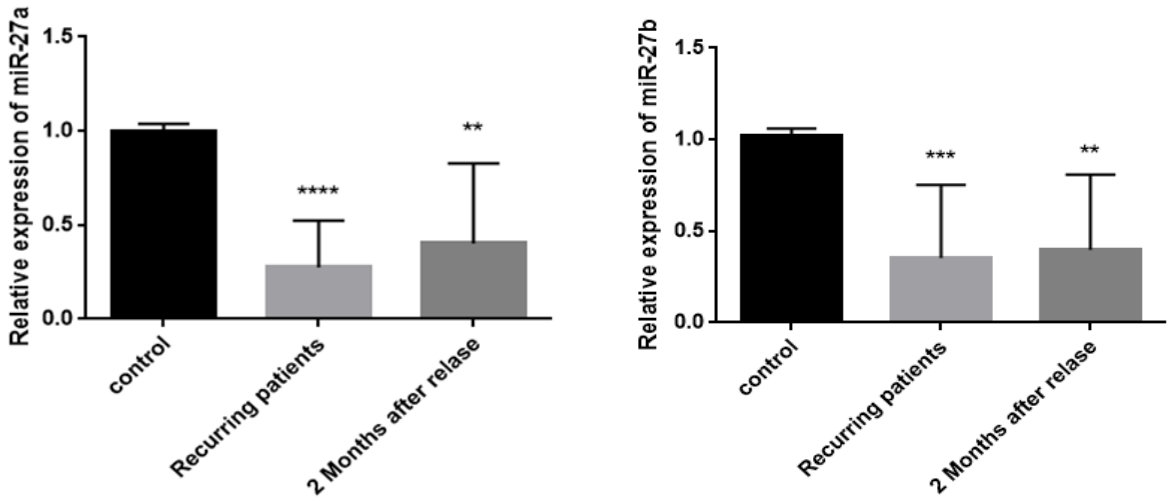


Figure 1. Relative expression of *miR-27a,* and *miR-27b* in three groups: recurring RRMS patients (n=30), two months after relapse patients (n=30), and controls (n=30). Relative quantitation for *miR-27a* (P<0.0001 and 0.003, respectively*)* and *miR-27b* (P<0.001 and 0.002, respectively) expression level were significantly lower in both recurrent and two months after relapse patients versus controls.

An earlier investigation using miRNA microarray analysis showed that *miR-27a* and *miR-27b* were down-regulated in peripheral blood of MS patient's subtypes including primary progressive, secondary progressive and relapsing-remitting disease (20). In another study, total RNA was extracted from $CD4^+$ T cells and miRNA expression patterns analyzed using Illumina-based small-RNA next-generation sequencing (NGS) in 12 secondary progressive MS (SPMS) and 12 controls. The results showed of miRNAs studied, *miR-27a-3p* ($p = 0.031$) and *miR-27b-3p* ($p = 0.031$) were found to be down-regulated in SPMS (21). Accordingly, *miR-27a* and *miR-27b* probably contributes to other autoimmune diseases with a similar immune pathologic mechanism. We observed *miR-27a* and *miR-27b* expression were significantly lower in both RRMS patients (recurrent and two months after relapse) than in the healthy subjects. The findings of our study indicated that *miR-27a* and *miR-27b* expression were statistically significant difference between groups studied and it also can be assumed these miRNAs associated with RRMS. Therefore, *miR-27a* and *miR-27b* probably contribute to MS or other autoimmune diseases pathological mechanism. MS disease as a poly-factorial illness could be various in different populations due to this fact that underlying genetic and environmental risk factors involved in this disease. However, more

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investigation on miRNAs expression are required in RRMS with diverse ethnical and with or no medicine to the best of knowledge of the role of *miR-27a* and *miR-27b* in pathogenesis of RRMS.

Conclusion

The data have shown the decreased rate of *miR-27a* and *miR-27b* transcripts in both RRMS patients groups (recurrence and at least two months after relapse). Thus, *miR-27a* and *miR-27b* may be predictive of treatment response. However, more cooperative studies are required to address the biomarker utility of these miRNAs.

Conflict of interest

The authors declare that they have no conflict of interest.

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