Iraqi JMS

Published by College of Medicine, Al-Nahrain University
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net/
Iraqi JMS 2025; Vol. 23(2)

Circulating MicroRNA-29b Expression as A Possible Prognostic Marker for Therapy Response in Multiple Sclerosis Patients

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Abstract

Background MicroRNAs (miRNAs) control the expression of targeted gene post-transcriptionally. Collecting evidences supports that miRNA dysregulation is related to the pathogenesis of autoimmune diseases.

Objective To assess the levels of microRNA-29b (miR-29b) as indicator of interferon beta and natalizumab therapy effectiveness in multiple sclerosis (MS) patients.

Methods One hundred and twenty blood samples were taken from patients at the MS Center in the Baghdad Medical City, during the period February to September, 2021. First sixty patients getting natalizumab (30 responders and 30 non-responders) and, the other sixty patients getting interferon beta (IFN-β) (30 responders and 30 non-responders). As well as, thirty normal volunteers were included in this study as control group. IFN-β and Natalizumab antibodies (Abs) were detected by using enzyme-linked immunosorbent assay (ELISA). On the other hand, Quantitative Polymerase Chain Reaction (qPCR) was used to assess the levels of miR-29b in blood samples of patients.

Results The concurrence of anti-IFN Abs in non-responder, responder MS patients and healthy control was (26, 16, 5) respectively. In addition, a high frequency of anti-natalizumab Abs in patients was found. The number of seropositive natalizumab Abs cases among the non-responder group, responder and healthy control was (5, 0, 0) respectively. On the other hand, there was a significant increase in microRNA-29b expression in patients' group with a median equal to 25584.00 compared to the control group (22476.50). The relation between the seropositivity of anti-IFN Abs and the level of microRNA-29b in IFN-β treated MS patients was highly significant with P value (<0.001). The study also showed a highly significant relation between the presence of anti-Natalizumab Abs and the level of miR-29b among Natalizumab-treated MS patients group (P value <0.001).

Conclusion The levels of miR-29b in the serum of the MS patients could be used as an informative indicator for the bioactivity of IFN-β and natalizumab treatment.

Keywords Interferon-β, multiple Sclerosis, microRNA-29b, Natalizumab

Citation Mohammed HA, Abbas AA, Shaheed NM. Circulating microRNA-29b expression as a possible prognostic marker for therapy response in multiple sclerosis patients. Iraqi JMS. 2025; 23(2): 303-311. doi: 10.22578/IJMS.23.2.13

List of abbreviations: ELISA = Enzyme-linked immunosorbent assay, IFN-β = Interferon beta, MicroRNAs = miRNAs, miR-29b = microRNA-29b, MS = Multiple sclerosis, qPCR = Quantitative polymerase chain reaction

Introduction

Multiple sclerosis (MS) is a recurrent chronic inflammatory disease of the central nervous system (CNS). During the disease course, incidents of CNS

inflammation ensue unpredictably. The principal cause of which remains undefined [1]. The involved factors in pathogenesis are generally grouped into three categories: immune factors, genetic association factors and environmental factors [2]. HLA-DRB1 * 15:01 consider as a genetic risk factor for MS susceptibility [3].

Interferons (IFNs) were the chief agents that reveal clinical proficiency in relapsing-remitting MS (RRMS). An INF beta (IFN-β) declines clinical relapses, decreases brain disease activity, and probably slows down the disability progression. The treatment may possibly induce the production of IFN-β antibodies in some patients. Such Abs bind to the IFN-β, and a subgroup of these binding antibodies (BAbs) is neutralizing antibodies (NAbs), which bind to IFN-β domains that are needed for receptor binding. Neutralizing Abs obstruct the bioactivity of injected IFN-β. The loss of IFN-β bioactivity is linked with reduced clinical efficiency of the drug and consequent worsening of the disease [4].

Natalizumab (Tysabri) is a humanized monoclonal Ab against the α4 chain of integrins and was the first targeted therapy to be permitted for the treatment of RR multiple sclerosis (RRMS). Natalizumab works as a selective adhesion molecule antagonist, which binds very late antigen (VLA)-4 and inhibits the translocation of activated VLA-4-expressing leukocytes across the blood-brain barrier (BBB) into the CNS [5]. Neutralizing Abs may also be formed during natalizumab (TYSABRI) therapy [6]. Neutralizing Abs lower the level of natalizumab in serum and, with constant presence, are related to a decrease in the therapy efficiency [7,8].

MicroRNAs (miRNAs) are a novel class of minor non-coding RNA molecules around 22 nucleotides long that control gene expression post-transcriptionally through binding to 3' untranslated region (UTR) of their mRNA targets, and leading to deprivation or transcriptional repression of the targeted mRNA. Upregulation the expression of microRNA-29B (miR-29b) in memory CD4+ T cells can imitate chronic T helper 1 (Th1) inflammation [9]. Surveillance of drug efficiency, is necessary to detect proper response to treatment. The presented work has been chosen due to the lack of previous Iraqi studies that handle the expression of different miRNAs in association with responsiveness to the treatment of MS patients.

Methods

This prospective study included one hundred and twenty patients suffering from MS who attending MS Center in Baghdad Teaching Hospital during the period February, 2021 to September, 2021. Their ages range was between 16-62 years (53 males and 67 females); the patients dividing into two groups: Sixty of them received natalizumab (tysabri) for approximately 1 year (30 responders and 30 non-responders), other sixty of them received betaferon for approximately 1 year (30 responders and 30 non-responders). All patients were diagnosed (according to MacDonald's criteria) and divided into responders and non-responders by consultant physicians (specialized neurologist).

This research was approved by the Institutional Review Board (20201099) in the College of Medicine at Al-Nahrain University.

Inclusion criteria

MS patients on treatment for about 12 months.

Exclusion criteria

MS patients with other chronic diseases.

The samples of the control group (30 individuals) had been collected from a Blood Donor Center in Al-Yarmuok Teaching Hospital and from apparently healthy volunteers, their age and sex matched the patients. All of them received no treatment with no complaints of other chronic or systemic diseases. Three (3) ml of whole blood was collected from all MS patients and the control group was put in a gel tube which was then centrifuged at 5000 rpm

for 5 min to get the serum. All the samples were preserved at (-20°C) till used. Enzyme-linked immunosorbent assay was used was for the detection of IFN-β and natalizumab Abs while quantitative polymerase chain reaction (qPCR) was used to assess the levels of miR-29b in blood samples of MS patients.

Statistical analysis

The statistical package for the social sciences (SPSS) version 26 was used for statistical analysis, categorical data were testified as counts and percentages, and the Chi-square test was used to describe the association between these data. The minimal statistically significant difference is less than or equal to 0.05.

Results

In this study, the age group 31-40 years was the largest one comprising 54 patients (45.0%) followed by <31 years' age group, which involved 47 patients (39.2%) and 19 (15.8%) patients within the age group >40 years. (Figure 1).

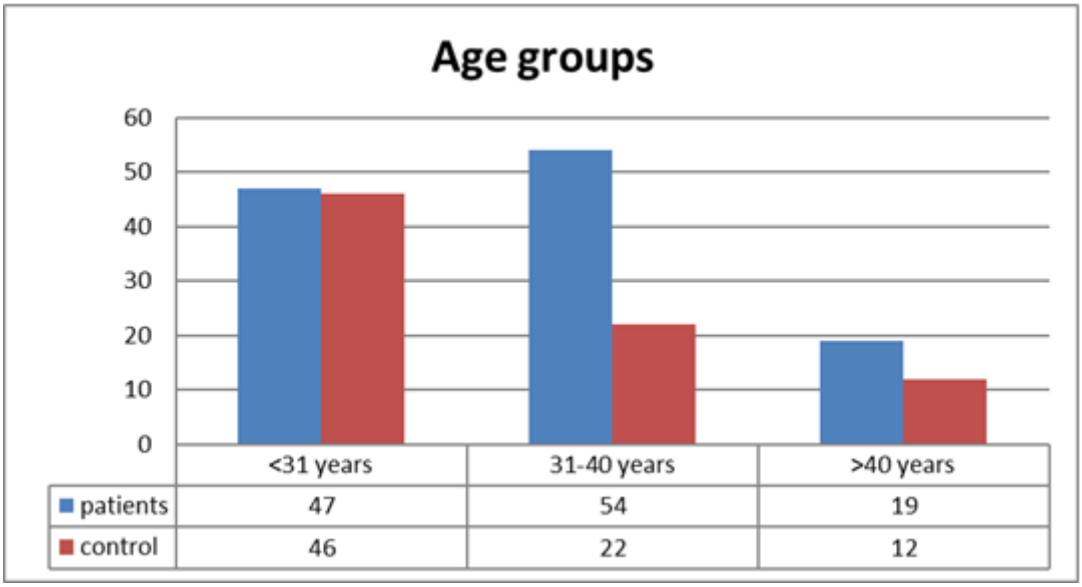

Figure 1. Age distribution of studied groups

The number of females patients involved in the present study was 67 while the number of males patients was 53 (Figure 2).

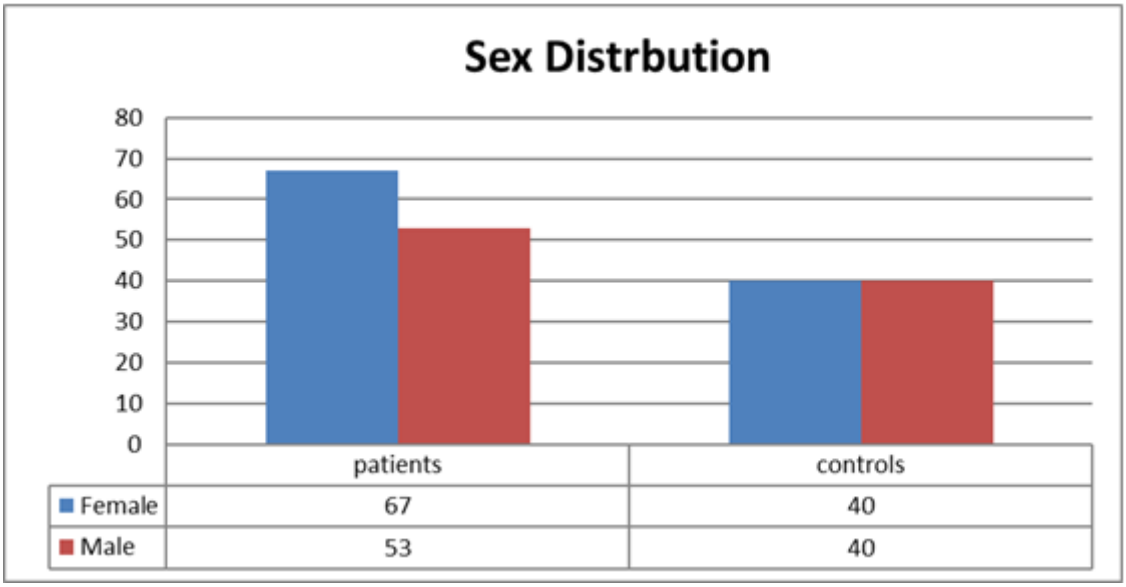

Figure 2. Sex distribution of studied groups

The proportion of anti-IFN Abs in non-responder, responder MS patients and healthy control was (26, 16, 5) respectively with statistically significant differences (Figure 3). While the number of positive natalizumab Abs cases among the non-responder group, responder and healthy control was (5, 0, 0) respectively (Figure 4).

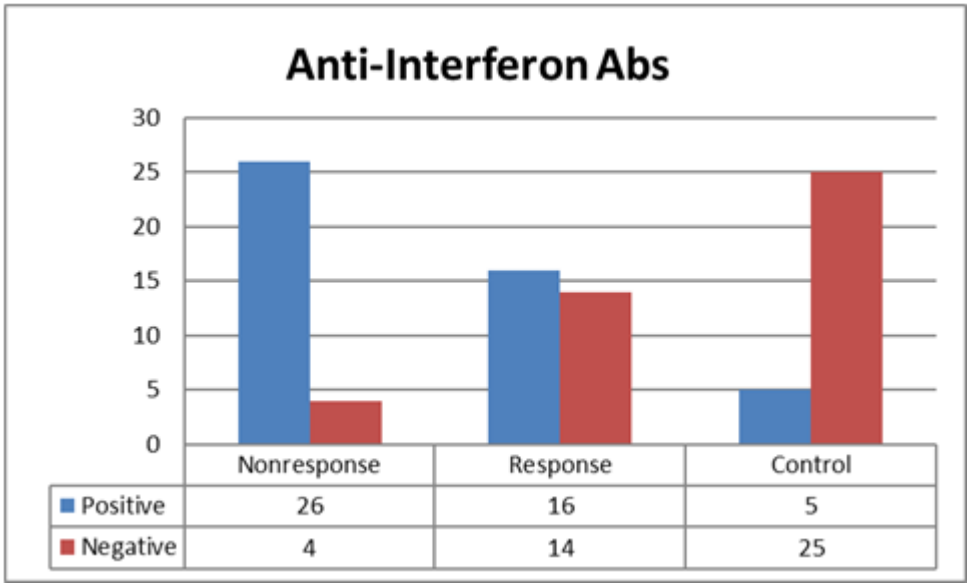

Figure 3. The prevalence of anti-interferon antibodies in MS patients and healthy control

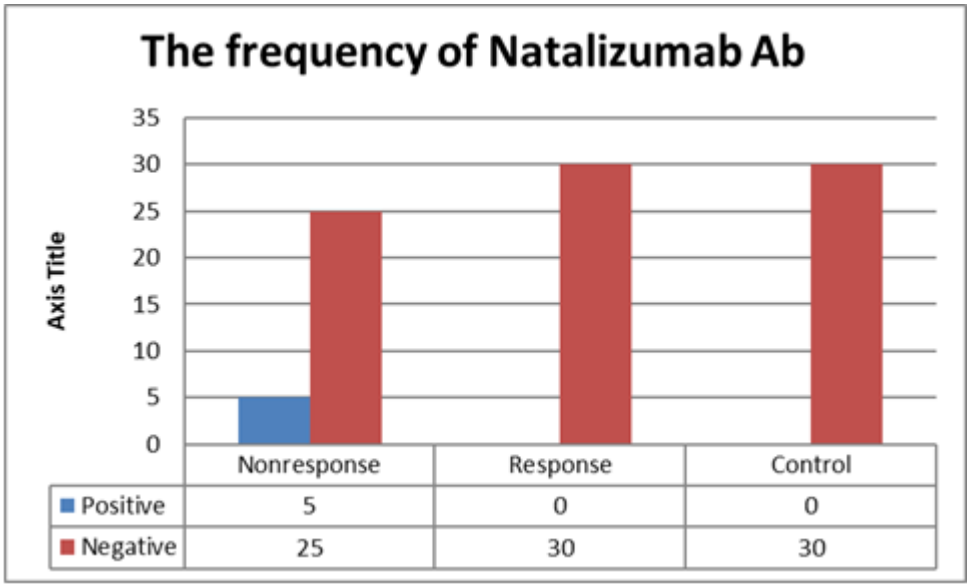

Figure 4. The prevalence of anti-Natalizumab Ab in MS patients and healthy control

Current results also exhibited a significant increase in miR-29b expression in the patients' group with a median equal to 25584.00 compared to the control group (22476.50) (Figure 5).

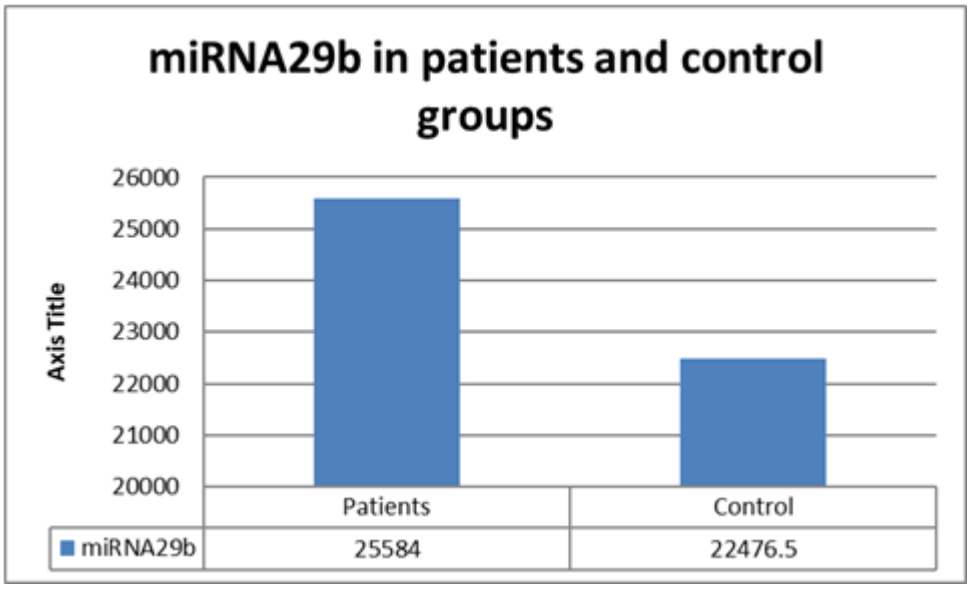

Figure 5. The micro-RNA-29b expression in studied groups

It was revealed in the present study a highly significant relation between the presence of anti-IFN abs and the level of miR-29b in IFN-treated MS patients with P value (<0.001) (Figure 6).

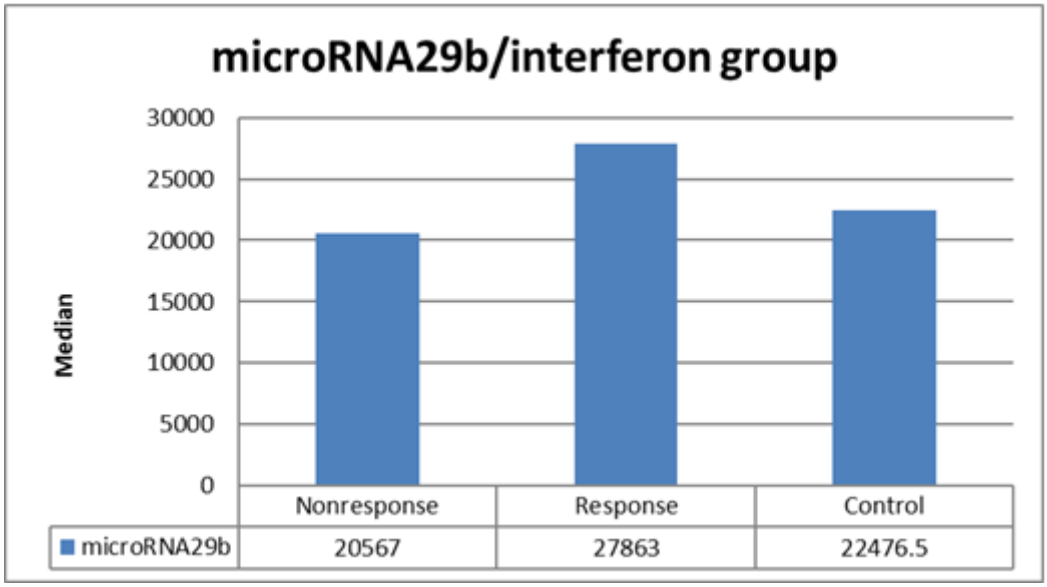

Figure 6. miR-29b expression in interferon-treated patients and control group

The study also showed a highly significant relation between the presence of anti-natalizumab Abs and the level of miR-29b among natalizumab-treated patients (P value <0.001) (Figure 7).

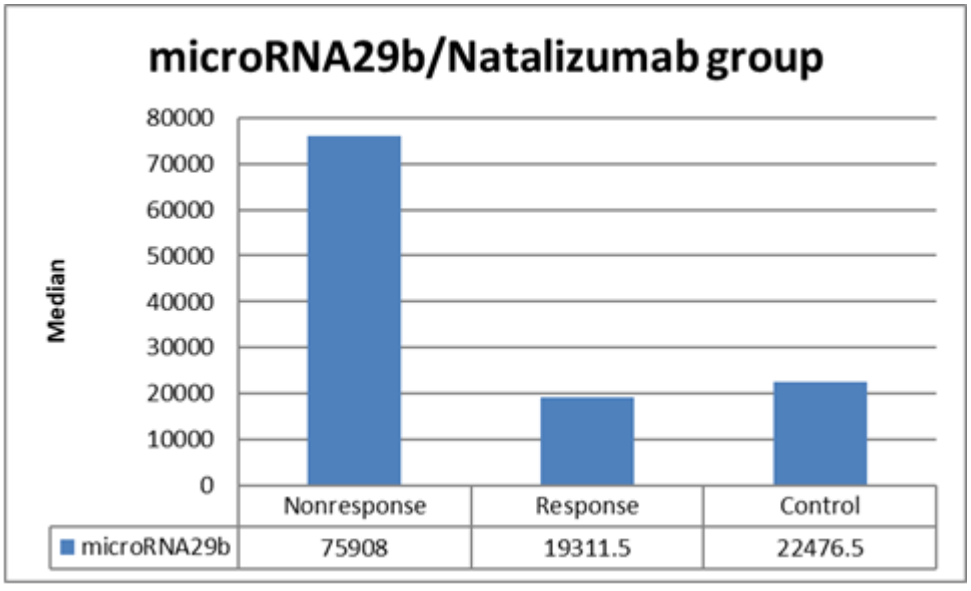

Figure 7. miR-29b expression in natalizumab treated patients and control group

Discussion

The current study evaluated the expression of miRNA of RRMS patients treated with IFN-β for one year to detect a possible association between of immune-modulatory treatment and the expression of miRNA. The expression pattern of miR-29b-3p was estimated by quantitative real-time PCR method in three groups (IFN-treated MS patients: non-responder, responder and apparently healthy control). Individuals treated with IFN-β have variable responses; the most essential aspect is the reduction in relapses and disability progression over time. The difficulty in monitoring response clinically lies in the fact that relapse rate naturally declines over time and disability progression, for the majority, is a process that develops slowly over many years (decades). Patients who continue to experience disease activity are non-responders or suboptimal responders, but it is difficult to know if an individual is responding to therapy and to what degree. Magnetic resonance imaging (MRI) is the most sensitive biomarker of subclinical MS disease activity that may continue in the absence of clinically apparent signs of progression. The numbers of new lesions predict future disability [10]; however, there is a lack of correlation between lesions and relapses at an individual level, suggesting a need for other biomarkers [11]. Present findings demonstrated that out of 60 MS patients, there were 42 patients who were positive for anti-IFN Abs (26 non-responding, 16 responding) representing 70% (P value <0.001) of the cases included in the current study. Fernández et al. reported that BAbs were positive in 32% of cases treated by IFN [12]. Aarskog et al. found that of the 827 patients, 363 (43.9%) had serum BAbs after more than 12 months of IFN-β treatment [13].

Other drug for MS besides the IFN-β is natalizumab. It is a monoclonal Ab directed against the α4 component of the α4β1 integrin (VLA4; CD49d) expressed on the surface of lymphocytes and monocytes [14]. In the current work, the number of positive natalizumab Abs cases among the non-responder group, responder and healthy control was (5, 0, 0) respectively. Previous study, reported that 57 patients receiving natalizumab (9%) had detectable abs at some time during the study (2 years). Of these 57 patients, persistent Abs to natalizumab were detected at ≥2 times that were ≥42 days apart developed in 37 patients (6%), who also had an increase in infusion-related adverse events and a loss of efficacy of natalizumab [15]. Other study showed that natalizumab Abs were detected in 58% of the natalizumab-treated patients. All patients developed their antibodies before week 24. The presence of Abs was inversely correlated with serum natalizumab concentration (P <0.001) [16]. According to 3 phase study, high baseline natalizumab anti-drug antibody (ADA) titers accurately predict persistency. Despite continuous treatment, the majority of patients with persistent ADA had no detectable drug levels indicating a loss of efficacy [17].

The present study revealed a significant increase in miR-29b expression in the patients group with a median equal to 25584.00 compared to the control group (22476.50), with significant relation between both the incidence of anti-IFN Abs and anti-natalizumab Abs with the level of miR-29b in MS patients with P value (<0.001). A previous study presented by Fattahi et al. indicated that the expression level of miR-29b-3p changed related to IFN-β response. Moreover, miR-29b-5p was downregulated under IFN-β treatment in responders versus non-responders [18]. On the other hand, according to prior study, ninety-seven percent of miRNA candidates including miR-29b identified by Next-generation sequencing were down-regulated in secondary progressive multiple sclerosis (SPMS) (P <0.05). [19]. miR-29b is a key part of a negative feedback loop that controls the balance of Th1 cells by repressing multiple target genes, including T-bet. The miR-29b expression has been shown to be up-regulated in memory CD4+ T cells from RRMS patients, which may reflect chronic Th1 inflammation. Persistent up-regulation of both miR-29b and IFN-γ in MS is indicative of chronic inflammation [20]. Functionally, changes in this specific miRNA inhibit Th2 cell differentiation by inhibiting B lymphoma Moloney murine leukemia virus (Mo-MLV) insertion region 1 homolog (BMI1)

and IL-4 expression, providing evidence that miRNAs influence T cell polarization. An antecedent study has identified miR-29b as an IFN-γ-inducible miRNA in CD4+ memory T cells, which acts in a negative feedback loop to control Th1 cell bias by inhibiting T-bet and IFN-γ transcription. In MS patients, the increase in miR-29b suggests a dysregulation of this feedback loop and an important factor that can bias Th1 cell differentiation [20].

The limitations of the study was the difficulty of obtaining the required samples, given the coincidence of the study with the COVID19 epidemic.

In conclusion, miR-29b could be a useful biomarker of treatment response in MS patients.

Acknowledgement

The authors would like to thank all the patients, family members and staff from all the units that participated in the study.

Author contribution

Dr. Abbas: Designed the research study, supervised this work and commented on the manuscript. Mohammed: Collected the data, wrote the manuscript with support from her supervisors. Dr. Shaheed: Provided patient samples and clinical assessment.

Conflict of interest

The authors declare no conflicts of interest.

Funding

No external funding was received for this research.

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Received Jul. 18th 2023
Accepted Sep. 11th 2023