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Profiles of miRNA expression and IFN-γ serum level as biomarker for the development of Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is associated with a wide spectrum of sensory, motor, and psychological disorders. Cytokines level and microRNA (miRNA) expression have roles in the disease's progression and the start of a damaging immune response in the central nerve system. This research study aimed to determine the role of interferon-γ (IFN-γ) and microRNA-326 (MiR-326) as prognostic factors for the development of MS disease in relation to different treatments. This case-control study included 100 participants, classified as 80 MS patients and 20 apparently healthy subjects as a control group. IFN-γ level was determined by an enzyme linked immunosorbent assay. The expression level of micR326 was determined by the reverse transcription polymerase chain reaction technique. The mean level of serum IFN-γ in MS patients (102.83 ± 15.79 ng/ml) was significantly higher than in the control group (61.25 ± 12.51 ng/ml) (p=0.001). A higher concentration of IFN-γ was observed in the secondary progressive form of MS disease relative to relapsing-remitting multiple sclerosis (RRMS) and in comparison, with the controls group, this IFN-γ cytokine level was significantly higher in treatment-naive patients. There was an increase in the mean fold change of miRNA-326 expression in patients (3.1 ±1.65) compared to the control group (1.03 ±0.23). In conclusion, secondary progressive multiple sclerosis (SPMS) has higher IFN-γ serum level than RRMS. MiR-326 may participate in the development of MS and its expression can be a useful biomarker for the prediction of MS.

Keywords: MS, IFN-γ, MiR-326.

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Introduction

Multiple sclerosis (MS) is an organ-specific, chronic inflammatory autoimmune disease in the central nervous system (CNS), results in disability and reduction of the quality life of infected people. It affects about 2.3 million people worldwide, generally younger than 50 years.[1]

There are two stages of MS, early inflammation response for relapsing-remitting MS (RRMS) and delayed deterioration of the neurons linked to non-relapsing progression, i.e. secondary progressive (SP) and primary progressive (PP) MS.[2] The most prevalent clinical form of MS is the RRMS, present in about 85% of all MS patients.[3]

Patients with RRMS may benefit from treatment. The majority of disease modifying drugs (DMDs) have immunosuppressive or immunomodulatory effects, these drugs target inflammation in patients reducing the risk of relapse and delaying progression.[4]

Numerous DMDs, including interferon β (IFN-β) which can be used in the management of MS.[5] Rituximab (RTX) is a chimeric monoclonal antibody that targets CD20 positive B cells, causing apoptotic cell-mediated death and reducing inflammation.[6]

The immunological response associated with MS is mediated through the production of cytokines and chemokines by CD4+ T cells.[7] Demyelinating lesions are formed in the early MS stages when invasive lymphocytes release pro-inflammatory mediators such as cytokines, which stimulate the central nervous system's innate immune system and attract more peripheral immune cells.[8] IFN-γ has inflammatory and aggravating effects on MS. Compelling evidence has shown that IFN-γ has dual effects in autoimmune disease.[9]

MicroRNA (MiRNA) is one of the epigenetic factors that has a role in immunological dysregulation and inflammatory autoimmune disorders. It is associated with different miRNA expression levels, alterations in the targeted tissues and cells of either innate or adaptive immunity.[10] MicroRNA-326 belongs to the miR-15/107 gene group, and neural stem-cell differentiation and brain development are linked to its expression. It has been reported that miR-326 has significant functions in autoimmune disease such systemic lupus erythematous and multiple sclerosis.[11] This study aimed to assess the miR-326 expression pattern and the blood level of INF-γ in MS patients in relation to the stage of disease and type of treatment.

Subjects and Methods

This case-control study included 100 participants, 80 patients with MS, and 20 normal subjects as a control group. Patients with different types of MS were collected from Al-Husseini Teaching Hospital in the holy city of Karbala, from November 2022 till end of April 2023.

A venous blood sample (2 ml) was collected from each study participant. Of these, an aliquot (1 ml) was put in a gel tube for serum separation. Separated serum was kept frozen at -20°C, until used for measurement IFN-γ level. The second aliquot (10 ml) of blood was transferred into an anticoagulant tube and immediately stored at -20°C for assessment of miR-326 by the reverse transcription polymerase chain reaction (RT-PCR).

The IFN-γ level was determined by enzyme linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, China), according to the manufacturer's instructions. The final ELISA products were read using a microtiter plate reader (Human Diagnostics, Germany).

For assessment of miR-326, total RNA was extracted from whole blood using the RNA purification kits (TransZol™, TransGen, China) according to the manufacturer's instructions. Primers for miR-326 and U6 calibrators were designed by a biotechnology company (Macrogen, South Korea). U6 expression was used for the normalization of miRNA expression. The RT-qPCR was carried out using an automated machine (RT-qPCR, Thermo Fisher Scientific, USA). The reaction was carried out using the master mix (1-Step RT-qPCR System, Promega, USA). The thermocycling conditions were: one cycle of reverse transcription at 37˚C for 15 minutes, then one cycle of RT inactivation at 95˚C for 10 minutes, followed by 40 cycles each included denaturation at 95˚C for 10 sec, then annealing at 60˚C for 30 sec and extension at 72˚C for 30 sec.

Gene expression (gene fold) value was calculated by the following equation[12]:

Relative quantity (RQ) =2 (-ΔΔCT)
First, the average CT (cycle threshold) value for each triplicated sample was collected using an RT-PCR instrument to calculate the gene fold, then ΔCT value determined as follows for every sample:

Δ CT = CT (tested miR326) – CT reference geneU6)
ΔΔCT=ΔCT (tested sample)-ΔCT (reference gene)
Fold gene expression RQ=2-(ΔΔCT)

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, IBM, version 26) was used for statistical analysis. The difference of qualitative results among different groups was determined using the Chi-square test. Results of IFN-γ level and miR326 are presented as arithmetic mean ± standard deviation (±SD). One-way ANOVA was used for comparison between patients and the control group. The receiver operating characteristic (ROC) curve was used for assessment of the overall diagnostic performance of a test. A $p<0.05$ was considered statically significant.

Results

In this study, females recorded higher frequency 60 (75%), having a 3:1 female to male ratio. The age of patients ranged from 18-63 and, the average age of patients with multiple sclerosis was 36.8 years and 35.1 years the average age of the control group. The frequency distribution of MS patients based on class of disease was 62 (77.5%) patients with RRMS and 18 (22.5%) with secondary-progressive multiple sclerosis (SPMS) as shown in Table 1.

Table 1. Distribution of research participants by different study parameters.

Study parameters	MS patients N=80 N (%)	Control Group N=20 N (%)
Sex		
Male	20 (25)	6 (30)
Female	60 (75)	14 (70)
Age		
range	18-63	19-57
Mean	36.8	35.1
MS Class		
RRMS	62 (77.5)	
SPMS	18 (22.5)	
Treatment types		
Betaferon	72.5 (58)	
Rituximab	17.5 (14)	
Without treatment	10 (8)	

N: number of cases; RRMS: relapsing-remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis

Evaluation of IFN-γ serum level in multiple sclerosis patients and the normal control group

In the current study there was a marked increase in the serum level of IFN-γ in MS patients. The results illustrated that serum levels of IFN-γ in MS patients was significantly higher compared to the control group (p=0.001), as shown in Figure 1.

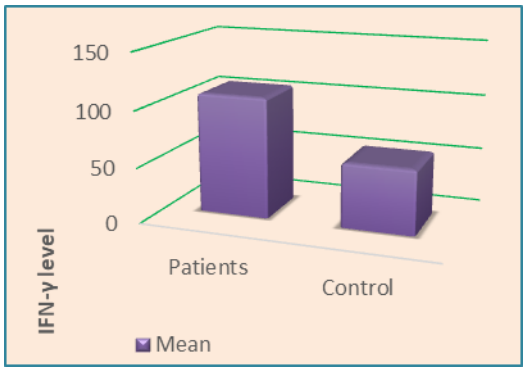

Figure 1. IFN-γ serum level in multiple sclerosis patients and the control group.

Estimation of IFN-γ serum level in multiple sclerosis patients according to the type of disease

In our study MS patients in SPMS stage exhibited an increased level of IFN-γ, in contrast to RRMS. The statistical analysis showed significant variations in the level of IFN-γ among the study groups as shown in Figure 2.

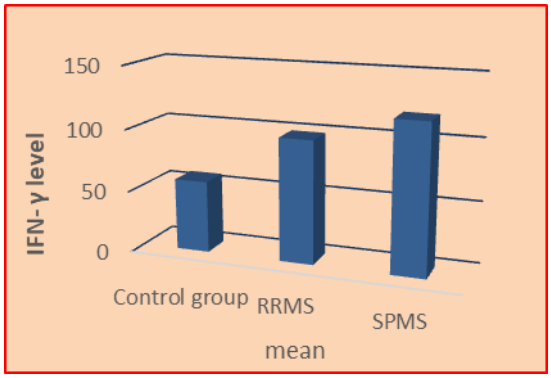

Figure 2. IFN-γ serum level in different types of multiple sclerosis patients. RRMS: relapsing remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis.

Evaluation of serum level of IFN-γ in multiple sclerosis patients according to type of treatment

The results in the current study showed that serum IFN-γ levels in patients receiving interferon-β treatment was significantly lower than MS patients who did not receive the treatment (p=0.001). Additionally, compared to the controls, the amount of this cytokine was significantly higher in treatment-naive patients (p=0.001) as indicated in Figure 3.

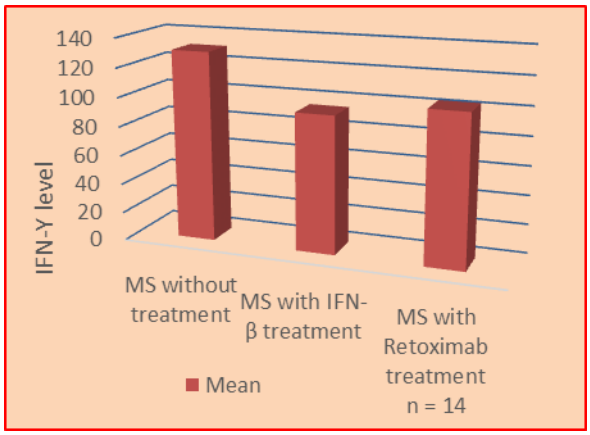

Figure 3. IFN-γ serum level in multiple sclerosis patients with different types of treatment.

MiRNA-326 expression in multiple sclerosis patients and controls

MiR-326 expression was remarkably increased in multiple sclerosis patients compared to controls (p< 0.001) as shown in Figure 4 and figure 5. By the ROC analysis, MS patients could be distinguished from controls. Expression of miR-326 gave at an area under the curve (AUV) value of 0.91, the sensitivity was 87.5%, and specificity 80%, Cut off value was 1.185, as illustrated in table 2 and figure 6.

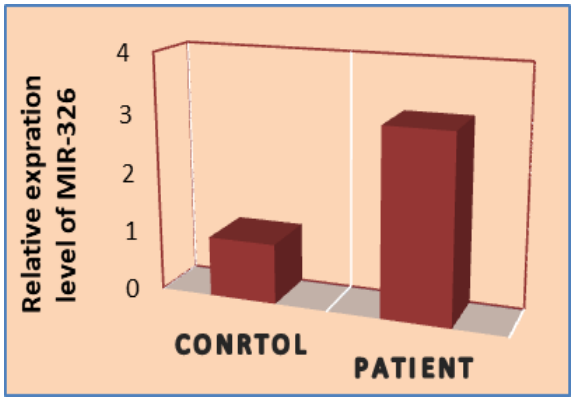

Figure 4. Comparison of the mean of the fold change difference of miR-326 between MS patients and controls.

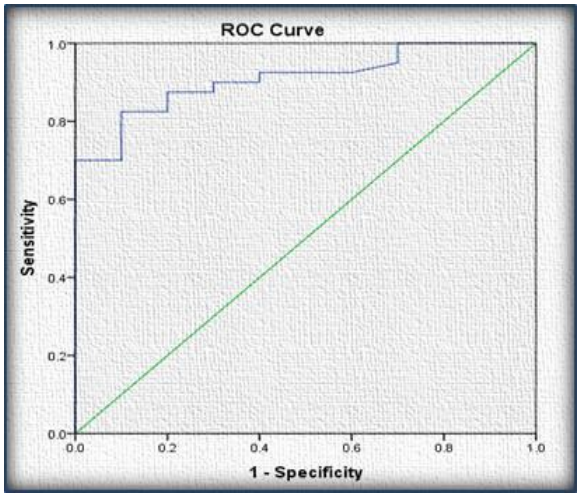

Figure 5. Receiver operating characteristic (ROC) curve for miRNA-326 in MS patients.

Table 2. Sensitivity and specificity of miRNA-326 in diagnosis of MS patients.

AUC	*p*-value	Cut off	Asymptotic 95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
0.91	0.001	1.185	0.826	0.992	87.5%	80%

AUC, area under the curve, and values were significant at $p < 0.05$.

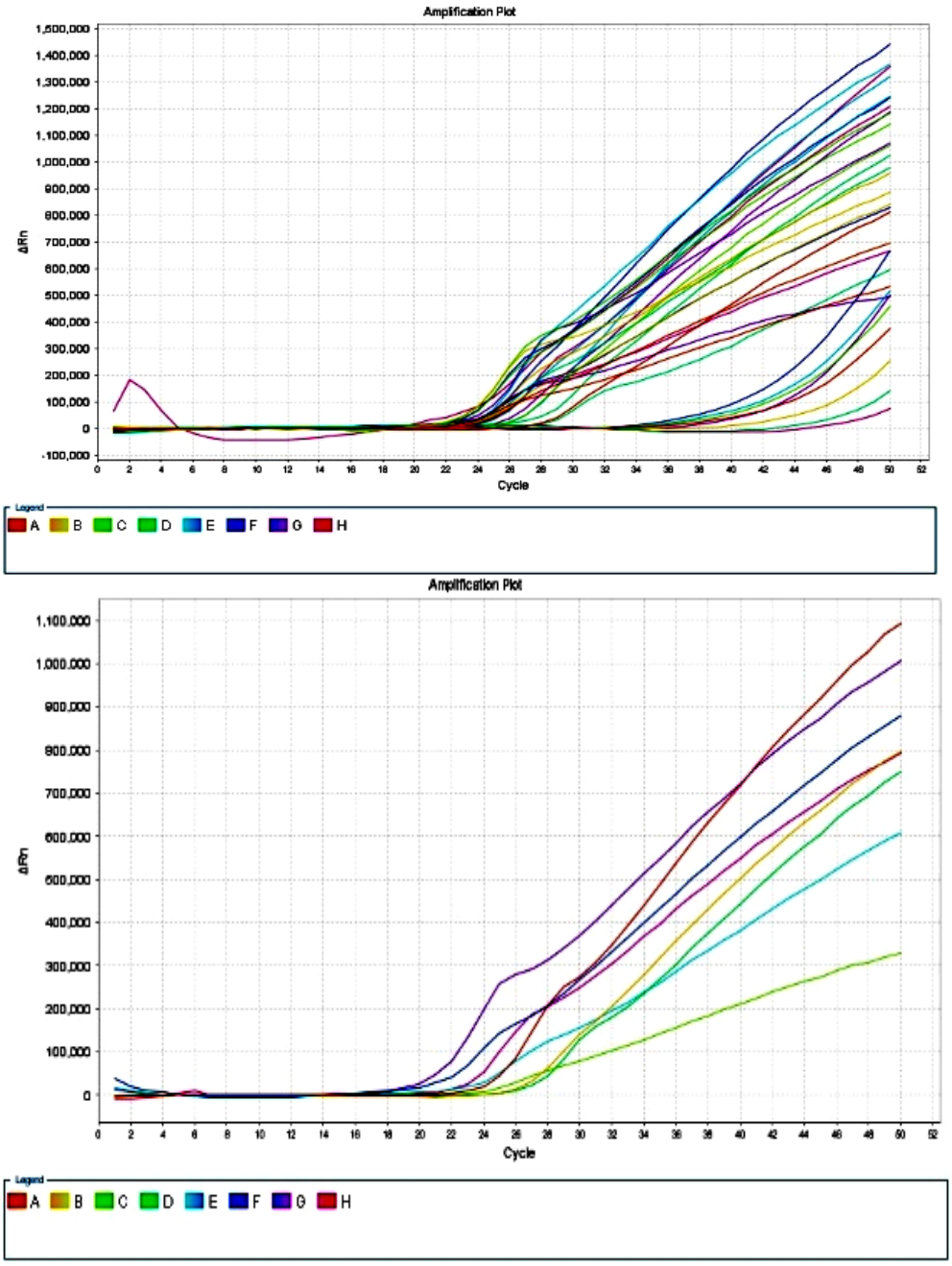

Figure 6. Real time PCR images showing the cycle threshold (Ct) value of selected microRNA genes.

Discussion

In the present study we observed that the majority of patients were of RRMS class and the minority of patients with SPMS table (1). This is in line with findings of Dias de Sousa, et al., 2022,[7] as they observed that the distribution of patients according to class of MS was 70% of patients with the RRMS and 20% of patients with SPMS while only one patient (3.33%) with primary-progressive multiple sclerosis (PPMS).

In the current study there was a marked increase in the serum level of IFN-γ in MS patients figure (1). This results agreed with those of the study by Talbot, et al., 2022,[15] who observed that patients with RRMS had higher cerebrospinal fluid (CSF) concentrations of IFN-γ, C-C motif chemokine 22 (CCL-22) and Interleukin 27 (IL-27) than the control group. Also, the study by Singh, et al., 2017,[16] found that patients with recently diagnosed RRMS have significantly higher levels of IFN-γ expression in their T cells as compared to controls. Also, the study by Abd et al., 2023,[17] reported that increased concentration of pro-inflammatory cytokines including IFN-γ and IL-17 lead to persistence inflammation and subsequent abnormal immune response that led to progressive disease. On other hand, elevated serum levels of IFN-γ were reported in MS patients relative to the control group.18 Additionally, higher levels of IFN -γ, IL-17, IL-6, tumor necrosis factor-α, IL-10 and IL-4 were observed in progressive MS compared to the control group.[19] Also, patients with the RRMS and SPMS types had higher frequencies of IL-17 positive or IFN-γ positive mononuclear cells in their peripheral blood.[20]

Another study by Kallaur, et al., 2013,[21] evaluated the pro-inflammatory cytokine serum levels in RRMS patients and assessed the relationship between the cytokine profile and the disease activity and progression. They found elevated serum IFN-γ, IL-12 and IL-4 levels in RRMS compared with the control group. The study by Kebir, et al., 2009,[22] found that lymphocytes obtained from the blood of relapsing MS patients were more likely to proliferate into T helper 17 (Th17) cells that produce IL-17, IFN-γ and other proinflammatory cytokines that disrupt tight junction proteins in the central nervous system (CNS) endothelial cells and considered as a potent pathogenic factor in disease immune-pathophysiology.

In our study MS patients in SPMS stage exhibited an increased level of IFN-γ, in contrast to RRMS as shown in Figure (2), these results agreed with those of Dias de Sousa, et al., 2022,[7] who found that the level of IFN-γ production by peripheral blood mononuclear cells from SPMS was significantly higher. They suggested that elevated IFN-γ level (the main cytokine that determines naive CD4+ T cells differentiate into Th1 lymphocytes) may be associated with clinical manifestations of more severely disabled patients.

Another study by Kallaur, et al., 2017,[19] compared the serum cytokine profile of patients with progressive MS and RRMS patients, and its correlations with the disability and disease progression with controls in a population from Southern Brazil. They found that IFN-γ levels in progressive MS were elevated compared to controls, and IFN-γ levels were higher in progressive MS than in RR-MS. Also, IFN-γ level tends to increase in remitting MS compared to relapsing RRMS patients and controls.[23]

The results in the current study showed that serum IFN-γ levels in patients receiving interferon-β treatment was significantly lower than MS patients who did not receive the treatment, and the amount of this cytokine was significantly higher in treatment-naive patients figure (3). This study findings are in line with those of Ganjia, et al., 2020,[24] who showed that serum levels of IFN-γ in treatment-naive patients were significantly higher than in the controls, and reduced levels of IFN-γ among patients treated with interferon beta-1 (IFN-β1). Also, these study data are consistent with those of the study by Guerrero-Garcia, 2016,[23] who found high serum level IFN-γ in treatment-naive RRMS patients than in controls and reduced levels of IFN-γ among patients receiving IFN-β1 treatment. Another research study by Trenova, et al., 2014,[25] observed higher serum levels of IFN-γ in RRMS patients without disease modifying treatment (IFN-β1) than the controls

and lower IFN-γ in RRMS treated patients with interferon- β -1b.

Our study findings show that MiR-326 expression was remarkably increased in multiple sclerosis patients compared to controls figure (4) and figure (5). The clinical significance of MiR-326 in the MS patients is described in table (2) and the receiver operating characteristics (ROC) curve figure (6) of miR-326 gave at an area under the curve (AUV) value of 0.91, the sensitivity was 87.5%, and specificity 80%.

This findings agreed with those of Basak and Majsterek, 2021,[26] who demonstrated that the expression level of miR-326, miR-141, miR-200a, miR-155 and miR-223 were elevated whereas miR-15b, miR-20b, miR-26a, and miR-30a were downregulated. Also, the imbalance between pro- and anti-inflammatory processes could be attributed to the dysregulation of these miRNAs. Since the targets of these miRNAs are linked to the control of Th1/Th17 and the differentiation of Treg cells. Also, the study by Baulina et al., 2018,[27] revealed a significant upregulation of miR-326 in remitting MS patients compared to relapsing MS patients and controls. Another study by Niwald, et al., 2017,[28] observed significant differences in miR-326 expression, with a significantly higher expression in RRMS compared to controls. Furthermore, peripheral blood mononuclear cells (PBMCs), brain white matter lesions from MS patients, and a mouse model all showed increased expression of miR-326.29 In the same direction the study by Lin et al., 2021,[30] confirmed that miR-326 was overexpressed in brain white matter lesions patients with MS. The study by Azimi et al., 2019,[31] found that mir-326a was over-expressed in MS patients in comparison to control individuals and they proved that microRNA-326 plays an important role in the pathophysiology of MS. The study by Junker et al., 2009,[32] confirmed that in patients with active multiple sclerosis, miR-326 was upregulated, and targets the CD47 molecule of brain's resident cells, which in turn, suppresses macrophage phagocytic activity, and accelerate myelin deterioration. Similarly, the study by Du et al., 2009,[33] demonstrated that miR-326, a negative regulator of Th17 differentiation, may cause T naïve cells to differentiate into Th17 cells, and consequently, the severity of MS will increase.

The study by Sonkoly & Pivarcsi, 2019,[34] confirmed that miR-326 regulates T-cell differentiation and mediates the immune response in MS as an immune-related miRNA. Also, the study by Honardoost et al., 2014,[35] observed significantly up-regulation of miR-326 in relapsing phase of multiple sclerosis patients compared with remitting phase (p=0.0001) and controls (p=0.0091). Also, the ROC curve analysis confirmed valuable and precise potential of miR-326 to discriminate between relapsing and remitting phases of multiple sclerosis with with 100% specificity and sensitivity, at a proposed optimum cutoff point. They confirmed the potential of miR-326 as a diagnostic biomarker to discriminate between relapsing and remitting phases of multiple sclerosis disease. The study by Nguyen et al., 2022,[36] demonstrated that miRNAs are dysregulated in major neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and suggested that miR-326 could be considered as promising biomarkers for their diagnostic and prognostic approaches.

In conclusion, findings of this study indicated that concentration of IFN-γ and expression of miRNA-326 may play key roles in immunopathogenesis of MS disease in regard to the stage of disease and type of treatments. Furthermore, by regulating their levels, complication or deterioration of the neurons caused by the disease may be avoided.

Author Contributions

The study's principal investigators were MFD and BMA. MFD proposed the topic of this research and designed the study. BMA collected the data. All authors contributed to preparing the final draft of the manuscript, revised the manuscript, and critically reviewed the intellectual contents. In addition, they have all read and approved the final manuscript and are responsible for its accuracy and integrity.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Kufa University (H K/1063, dated October 2022).

Informed consent

A signed consent form was obtained from each study participant.

References

1. Mora, P and Chapouly, C. (2023). Astrogliosis in multiple sclerosis and neuro-inflammation: what role for the notch pathway? *Frontiers in Immunology*, 10.3389/fimmu.2023.1254586

2. Greenfild AL & Hauser SL. (2018). B-cell therapy for multiple sclerosis: entering an era. *Ann Neurol*; 83:13-26.

3. Wiśniewska, Klaudia, Ostańska, Agnieszka, Szafran, Adrianna, et al. (2023). Multiple sclerosis: A review of recent advances in diagnostics and treatments. Quality in Sport.; 14(1):57-64.

4. Wingerchuk DM, Weinshenker BG. (2016). Disease Modifying Therapies for Relapsing Multiple Sclerosis. BMJ 354: i3518.

5. Ebrahimimonfared M., Ganji A., Zahedi S., et al. (2018). Characterization of regulatory T-cells in multiple sclerosis patients treated with interferon beta-1a, CNS Neurolog. Dis. Drug Targets 17 (2): 113–118.

6. Chisari, C. C.; Sgarlata, E; Arena, S.; et al. (2022). Rituximab for the treatment of multiple sclerosis: a review*: Journal of Neurology* 269:159–183.

7. Dias de Sousa, M.A.; Desidério, C.S.; da Silva Catarino, J. et al. (2022). Role of Cytokines, Chemokines and IFN-+ IL-17+ Double-Positive CD4+ T Cells in Patients with Multiple Sclerosis. *Biomedicines*, 10, 2062.

8. Machado-Santos J, Saji E, Troscher AR, et al (2018). The compartmentalized inflammatory response in the multiple sclerosis brain is composed of tissue resident CD8+ T lymphocytes and B cells. *Brain Oxford Academic* 141:2066–2082

9. Hussein D and Darweesh M (2023). Role of EBV infection in Type-1 Diabetic nephropathy pathogenesis related to IL-12 level in patients. *BIO Web of Conferences*. 65(05041).

10. Alfadhl AZ, and Mayyada F Darweesh MF. (2024). Investigating the possibility of using miR-146a as a prognostic marker for breast cancer and its association with IL-12 in progressive disease. *MJBL* 21(2).

11. Zhao N.; Zou1 H.; Qin J.; Fan C et al. (2018). MicroRNA-326 contributes to autoimmune thyroiditis by targeting the Ets-1 protein. *Endocrine,* 59:(4-5).

12. Wang X., Zhou H., Cheng R., et al. (2020). Role of miR-326 in neonatal hypoxic ischemic brain damage pathogenesis through targeting of the δ-opioid receptor. *Molecular Brain*, 13:51.

13. Reyes-Mata *et al*. (2023). Global DNA Methylation and Hydroxy methylation Levels in PBMCs Are Altered in RRMS Patients Treated with IFN-β and GA—A Preliminary Study. *Int. J. Mol. Sci*, *24*(10), 9074

14. Yusuf F.L.A., José M. A. Wijnands, Mohammad Ehsanul Karim, et al. (2022). Sex and age differences in the Multiple Sclerosis prodrome. *Frontiers in Neurology*, 03 Nov 3:13:1017492.

15. Talbot J. Chow H.H., Mahler M., Buhelt S., et al. (2022). Relationship between cerebrospinal fluid biomarkers of inflammation and tissue damage in primary progressive multiple sclerosis. *Multiple Sclerosis & Related Disorders.* 68: 104209.

16. Singh AK, Novakova L, Axelsson M, et al. (2017). High interferon-γ uniquely in Vδ1 T cells correlates with markers of inflammation and axonal damage in early multiple sclerosis. *Front Immunol* 8:260.

17. Abd, S. A.; Darweesh, M. F.; Al-Muhanna, S. G. (2023). Investigation on Bacterial Infection, IL-17 and Some Biomarkers Related to COVID-19 Patients' Outcome. *Latin American Journal of Pharmacy*; 42(Special Issue):350-355.

18. Arellano, G., E. Acuña, L.I. Reyes, P.A. Ottum, et al. (2017). Th1 and Th17 cells and associated cytokines discriminate among clinically isolated syndrome and multiple sclerosis phenotypes. *Front. Immunol.* 8: 753.

19.-Kallaur, A.P.; Oliveira, S.R.; Simao, A.N.C.; et al. (2017). Cytokine Profile in Patients with Progressive Multiple Sclerosis and Its Association with Disease Progression and Disability. *Mol. Neurobiol.* 54: 2950–2960.

20. Huber, A.K.; Wang, L.; Han, P.; et al. (2014). Dysregulation of the IL-23/IL-17 axis and myeloid factors in secondary progressive MS. *Neurology*, 83, 1500–1507.

21. Kallaur A.P, Oliveira SR, Colado Simão AN et al (2013). Cytokine profile in relapsing-remitting multiple sclerosis patients and the association with the progression and the activity of the disease. *Mol Med Reports,* 7:1010–1020

22. Kebir H, Ifergan I, Alvarez IJ, et al. (2009). Preferential recruitment of interferon gamma-expressing Th17 cells in multiple sclerosis. *Ann Neurol.* 66:390–402.

23. Guerrero-Garcia, J.; Castaneda-Moreno, V A; Torres-Carrillo, N; et al. (2016). Interleukin-17A Levels Vary in Relapsing-Remitting Multiple Sclerosis Patients in Association with Their Age, Treatment and the Time of Evolution of the Disease. *Neuroimmunomodulation;* 23:8–17

24. Ganjia, A; Monfaredc, M E; Shapoorib, S; et al. (2020). Effects of interferon and glatiramer acetate on cytokine patterns in multiple sclerosis patients. *Cytokine,* 126: 154911

25. Trenova, A G; Slavov, G S; Manova M G' et al. (2014). Cytokines and Disability in Interferon-b-1b Treated and Untreated Women with Multiple Sclerosis. *Arch Med Res,* 45(6):495-500.

26. Basak, J., & Majsterek, I. (2021). miRNA-Dependent CD4(+) T Cell Differentiation in the Pathogenesis of Multiple Sclerosis*. Mult. Scler. Int.* 2021:8825588.

27. Baulina N., Kulakova O., Kiselev I, et al. (2018). Immune-related miRNA expression patterns in peripheral blood mononuclear cells differ in multiple sclerosis relapse and remission. *Journal of Neuroimmunology*.

28. Niwald M, Migdalska-Sęk M, Brzeziańska-Lasota E, et al. (2017) Evaluation of selected microRNAs expression in remission phase of multiple sclerosis and their potential link to cognition, depression, and disability. *J Mol Neurosci,* 63:275-282.

29. Ma X.; Zhou J.; Zhong Y.*;* et al. (2014). Narendra Singh, Mitzi Nagarkatti and Prakash Nagarkatti :2014 Expression, Regulation and Function of MicroRNAs in Multiple Sclerosis*: International Journal of Medical Sciences*; 11(8): 810-818.

30. Lin LN, Zhang QM, Ge YY, et al. (2021). A Review of miR-326 and Female Related Diseases. *Acta Histochem Cytochem.* Jun 29; 54(3):79-86.

31. Azimi, M., Ghabaee, M., Naser Moghadasi, A., et al. (2019). Altered Expression of miR-326 in T Cell-derived Exosomes of Patients with Relapsingremitting Multiple Sclerosis. *Iran. J. Allergy Asthma Immunol.* 18, 108–113.

32. Junker A, Krumbholz M, Eisele S, et al. (2009). MicroRNA profiling of multiple sclerosis lesions identifies modulators of the regulatory protein CD47. *Brain*; 132(12):3342-52.

33. Du C, Liu C, Kang J, et al. (2009). MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. *Nat Immunol*; 10(12):1252-9

34. Sonkoly, E. & Pivarcsi, A. (2019). microRNAs in Inflammation. *Int. Rev. Immunol.* 28; 535–561.

35. Honardoost MA, Kiani-Esfahani A, Ghaedi K, et al. (2014). miR-326 and miR-26a, two potential markers for diagnosis of relapse and remission phases in patients with relapsing-remitting multiple sclerosis. *Gene.* Jul 10; 544(2):128-33.

36. Nguyen TPN, Kumar M, Fedele E, et al. (2022). MicroRNA Alteration, Application as Biomarkers, and Therapeutic Approaches in Neurodegenerative Diseases. *Int J Mol Sci.* 23(9):4718.