

Comparative Analysis of IL-18 Expression Across Therapeutic Modalities in Multiple Sclerosis

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ARTICLE INFO

Received: Apr. 27, 2026

Revised: Jul. 23, 2026

Accepted: Jul. 30, 2026

Published: Sep. 30, 2026



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ABSTRACT: Background: Multiple sclerosis represents a chronic autoimmune pathology associated with immune-related demyelination and neuroinflammation in the central nervous system. Recently, interleukin-18 appears to represent one of the major cytokines among other inflammatory mediators, responsible for innate and adaptive immunity cross-talk and involvement in disease development. Its behavior in diverse therapeutic regimes remains not sufficiently studied. **Objective:** The aim of this work was to evaluate serum interleukin-18 concentration in multiple sclerosis patients under different treatments and assess its diagnostic value in terms of treatment efficacy and pathogenesis. All the subjects involved totaled 180, including 16 untreated patients, 20 negative control groups, and patients under 60 on first 48, second 36, and third-line therapies. **Methods:** Measurement of serum interleukin-18 concentration was performed using the enzyme-linked immunosorbent assay method. Cytokine levels were correlated with clinical variables, such as disability score, disease duration, and relapse frequency. Data analysis was done using the software package SPSS. There were certain differences found in interleukin-18 levels in distinct groups, suggesting its biomarker role as a marker of disease dynamics and therapy efficiency. To further explore clinical implications of our findings, further studies are required. **Results:** Significant differences in interleukin-18 levels were demonstrated among distinct groups. Untreated patients had moderate elevations, and healthy controls had the lowest levels. First-line therapies showed heterogeneous effects; some treatments were associated with lower cytokine levels and others with higher expression. The highest levels of interleukin-18 were found in patients receiving second-line treatments, followed by third-line treatments. Cytokine levels increased progressively with escalation of therapy and longer duration of disease. Correlation analysis revealed that disability scores (EDSS) was significantly associated with disease duration, relapse frequency, and disease severity, but not with serum IL-18 levels. Receiver operation characteristic analysis indicated moderate diagnostic ability. **Conclusions:** In summary, interleukin-18 indicates both the inflammatory burden and the immune modulation induced by therapy in multiple sclerosis. While it is not appropriate as an independent diagnostic marker, it may function as an adjunct biomarker for assessing disease activity and immunological variations during treatment regimens.

KEYWORDS: Multiple sclerosis; Interleukin-18; Cytokines; Disease-modifying therapy; Neuroinflammation

INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system characterized by immune-mediated destruction of the myelin sheath surrounding neuronal axons in the brain and spinal cord. This demyelination disrupts neuronal signal transmission, leading to progressive neurological dysfunction and reduced quality of life [1]. MS is a leading cause of non-traumatic disability in young adults, typically affecting individuals between 20 and 40 years of age, with a rising

global incidence that increases its socioeconomic burden [2], [3]. From a clinical perspective, the condition manifests itself heterogeneously from mild symptoms such as sensory dysfunction to serious disabilities such as mobility impairment, visual problems, and speech impairment [4]. The progression of the illness follows an episodic pattern, characterized by episodes of relapse followed by periods of remission, which ultimately lead to the accumulation of disability [5]. Despite the vast range of therapies used, the mechanism of action is diverse for each disease-modifying therapy (DMT) since they target various parts of the immune system with variable effectiveness among patients [4], [6]. Among the cytokines implicated in MS pathogenesis, Interleukin-18 (IL-18) has emerged as a key regulator of innate and adaptive immunity, promoting T helper 1 (Th1) and T helper 17 (Th17) responses, as well as amplifying inflammatory cascades through inflammasome activation [7], [8]. Elevated IL-18 levels have been reported in MS patients and are associated with disease activity and progression, suggesting a potential role as both a biomarker and a mediator of neuroinflammation [9]. Nevertheless, how each therapeutic approach regulates IL-18 secretion is still not well understood. Interleukin-18 (IL-18), one type of IL-1 cytokine that is predominantly secreted from activated macrophages, microglia, and dendritic cells and plays an important role in immunomodulation [7]. The treatment of MS has been revolutionized through the advent of DMTs targeting various aspects of the immune system, including immunomodulation, sequestration, and depletion of immune cells. While these treatments have proved to be effective in reducing relapse rates and disease progression, their effects on cytokine regulation and especially on IL-18 production are not yet fully understood [10], [11]. In MS, disease-modifying therapies are employed using different immunologic mechanisms. Interferon beta medications, which include Rebif, Avonex, and Betaferon, are first-line treatments that have mostly immunomodulating actions by decreasing proinflammatory reactions and modulating the functions of immune cells [4], [6]. Second-line treatments, including Natalizumab (Tysabri) and Fingolimod (Gilenya), decrease immune cell migration and trafficking, which results in the limited access of these cells to the central nervous system [4], [6]. Third-line therapies that include Ocrelizumab (Ocrevus) and Rituximab decrease immune cells via targeting CD20-positive B cells [4], [12].

Given the central role of IL-18 in linking innate immune activation to adaptive immune responses, evaluating its expression across therapeutic modalities may provide critical insights into treatment-specific immunological reprogramming and assess its potential as a biomarker of therapeutic response and disease activity. Such insights may refine our understanding of MS immunopathogenesis and inform the development of more precise, mechanism-based treatment approaches. Thus, the current study was performed in order to assess serum levels of IL-18 in patients with MS under different DMT, as well as to study the association between IL-18 expression, the type of therapy, and clinical factors. Also, the use of IL-18 as a biomarker for disease activity and treatment efficacy was evaluated.

MATERIALS AND METHODS

Peripheral blood samples (270) were collected from patients at the MS Centre at Baghdad Teaching Hospital during the period from November 2025 to February 2026, of which 160 samples met the inclusion criteria, which included defining the age group between 19 and 46 years, and strict exclusion criteria were also applied to reduce confounding factors for 110 patients, who were those with associated chronic or autoimmune diseases and contagious diseases, especially the John Cunningham (JC) virus, in addition to pregnancy cases, as well as individuals who have received modern treatments or are still under their influence. The included patients were classified according to the lines of treatments used into three main categories: first-line therapies comprising interferon beta formulations (Betaferon, $n = 20$; Rebif, $n = 20$; and Avonex, $n = 20$), second-line therapies including Natalizumab (Tysabri, $n = 24$) and Fingolimod (Gilenya, $n = 24$), and third-line B-cell-depleting therapies consisting of Ocrelizumab (Ocrevus, $n = 18$) and Rituximab ($n = 18$). The clinical sample was dominated by the relapsing-remitting MS disease with limited inclusion of cases of primary progressive MS in the group of patients treated with Ocrevus. Due to the limited availability of patients who had not undergone any previous treatment. An untreated/discontinued-treatment MS group was included, comprising 16 patients who were not receiving any DMT at the time of sample collection. To minimize the residual effects of previous medications, participants were required to have discontinued corticosteroid treatment for at least one month and immunomodulatory or immunosuppressive therapies for at least one year before enrollment.

The blood sample was left at room temperature for 10 minutes to allow clotting to occur, then the serum was separated using centrifugation at 2000 rpm for 15 minutes. After that, the samples were stored in a freezer at -20°C until used. The study protocol received ethical approval from the ethics committee at Mustansiriyah University/college of science (Ref.: BCSMU/1025/0012B), and the written informed consent of each participant and blood sample donor was obtained. Interleukin

IL-18 was evaluated using the enzyme-linked immunosorbent assay technique using a diagnostic kit from Sunlong Biotech (China). Its level was expressed as pg/ml, with a sensitivity of 0.5 pg/ml. Statistical analyses were performed using SPSS Statistics version 20. Continuous variables were expressed as mean $\pm$ standard deviation (SD), and statistical significance was established at a p-value of < 0.05 . To evaluate the diagnostic and prognostic performance of IL-18, receiver operating characteristic (ROC) curve analysis was performed. The area under the ROC curve (AUC), sensitivity, specificity, and optimal cutoff values were determined to assess the ability of IL-18 to discriminate between healthy and disease states. The clinical assessment of disease severity and expanded disability status scale (EDSS) was based on the specialist neurologists at the time of blood sample collection. Correlations between immunological and clinical parameters were assessed using Spearman's rank correlation coefficient (r). Correlation coefficients ranged from -1 to $+1$, indicating the strength and direction of the associations. The correlation matrix was visualized using heatmaps generated with the Seaborn library.

RESULTS AND DISCUSSION

The demographic and clinical characteristics of the study participants are summarized in Table 1. The cohort included negative and positive controls together as well as patients receiving first-, second-, and third-line treatments. Age distribution differed across groups, with younger individuals observed in untreated controls and the Tysabri group (second line). By contrast, high mean ages were evident among patients receiving first-line interferon-based therapies and were most pronounced in those undergoing third-line treatment with Ocrevus, indicating an enrichment of older individuals in more advanced treatment strata. The observed age pattern suggests a progression from younger, untreated individuals to relatively older patients receiving advanced lines of therapy, supporting the concept that treatment escalation in MS is often associated with increasing patient age, cumulative disease burden, and previous exposure to treatment [13], [14]. The age gradations usually reflect the choice of treatment and the course of the disease; patients may continue to use older, less effective treatment strategies or begin them due to differences in comorbidities, physician/patient preferences, or risk tolerance, which is most important. Delayed, highly effective treatment is often initiated after a history of inadequate disease control (previous inflammatory activity or worsening disability); this is consistent with the practical reality of this study.

Table 1. The demographic and clinical characteristics of the studied groups

	Groups	Age	Sex	Duration	EDSS	Severity	BMI	No. of relapses
Control groups	Negative control (20)	31.50 $\pm$ 0.82	M=8, F=12	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	25.39 $\pm$ 4.04	0.00 $\pm$ 0.00
	Positive control (16)	25.88 $\pm$ 5.86	M=2, F=14	2.75 $\pm$ 4.06	1.22 $\pm$ 1.21	3.50 $\pm$ 0.73	23.47 $\pm$ 2.70	0.88 $\pm$ 0.62
First line treatment	Rebif (20)	30.40 $\pm$ 7.23	M=4, F=16	2.20 $\pm$ 4.44	0.60 $\pm$ 0.60	2.70 $\pm$ 0.80	25.28 $\pm$ 3.23	1.20 $\pm$ 1.01
	Avonex (20)	34.70 $\pm$ 6.74	M=2, F=18	4.80 $\pm$ 4.18	0.85 $\pm$ 0.76	2.60 $\pm$ 0.82	28.63 $\pm$ 3.21	1.30 $\pm$ 0.47
	Beta-feron (20)	34.30 $\pm$ 8.75	M=8, F=12	7.50 $\pm$ 4.90	1.20 $\pm$ 0.77	2.60 $\pm$ 0.94	25.77 $\pm$ 4.28	1.70 $\pm$ 2.00
Second line treatment	Tysabri (24)	25.75 $\pm$ 5.77	M=6, F=18	4.83 $\pm$ 3.31	0.83 $\pm$ 0.70	2.63 $\pm$ 0.86	25.36 $\pm$ 3.48	2.00 $\pm$ 1.10
	Gilenya (24)	34.92 $\pm$ 6.93	M=6, F=18	7.33 $\pm$ 7.14	1.29 $\pm$ 0.99	3.00 $\pm$ 0.83	27.42 $\pm$ 5.65	2.25 $\pm$ 1.39
Third line treatment	Rituximab (18)	32.89 $\pm$ 6.94	M=2, F=16	9.11 $\pm$ 4.32	1.17 $\pm$ 0.97	2.78 $\pm$ 1.46	26.02 $\pm$ 4.20	2.56 $\pm$ 1.46
	Ocrevus (18)	38.78 $\pm$ 5.06	M=8, F=10	7.11 $\pm$ 4.74	1.39 $\pm$ 0.99	4.22 $\pm$ 0.81	25.13 $\pm$ 3.70	1.56 $\pm$ 0.86

The negative control group showed no clinical disease, whereas the positive control group exhibited mild disability and low relapse activity. In the treated groups, first-line treatments were associated

with a relative decrease in EDSS scores and moderate relapse rates, despite some variability in disease duration. Second-line treatments showed a trend toward increasing disease burden with higher disability scores and relapse frequency. This pattern was most pronounced in the third-line treatment groups, which showed the highest clinical severity scores and relapse rates. Body mass index remained similar across all groups. Overall, disease severity and the relapse burden increased progressively with treatment escalation, consistent with their use in more advanced or refractory disease. The progressive increase in EDSS from untreated and first-line groups to second- and third-line therapies supports the concept that treatment escalation in MS is closely associated with accumulating neurological disability and disease burden [15], [16].

Some may tend to interpret the low EDSS score and moderate relapse rates in the group of patients who received first-line treatment as superior efficacy of interferon, and these patients may be suffering from inactive, slow-progressing disease when treatment begins, and this would lead to a decrease in the severity of the EDSS score and a reduction in relapses, even if the biological effect of interferon is modest compared to subsequent treatments [16]. It is important to note that the noticeable increase in the rate of relapses and disability with the escalation of treatment is due to worsening disease activity while receiving previous treatments, which leads to the accumulation of disability driven by underlying neurodegenerative processes. Therefore, patients in the advanced stages of treatment have high degrees of EDSS despite varying relapse rates. This is what was found in the Ocrevus group, where the severity of disability remains high even when the number of relapses is relatively lower than in some other treatments. Interleukin-18 (IL-18) levels differed markedly across study groups, as shown in Table 2, demonstrating a treatment-dependent pattern of inflammatory activity. The lowest concentrations were observed in healthy controls (399.74 ± 140.35), whereas untreated patients (576.25 ± 311.26) exhibited moderate elevations, indicating increased baseline inflammation.

Table 2. Interleukin-18 concentration in the studied groups

	Groups	Symbol	Mean $\pm$ SD	Significancy
Control groups	Negative control	a	399.74 $\pm$ 140.35	b,d,e,f,g,h,i
	Untreated patients	b	576.25 $\pm$ 311.26	a,d,e,f,g,h,i
First line treatment	Rebif	c	473.59 $\pm$ 149.64	d,e,f,g,h,i
	Avonex	d	819.94 $\pm$ 313.88	a,b,c,f,g
	Beta-feron	e	891.77 $\pm$ 185.44	a,b,c,f,g
Second line treatment	Tysabri	f	1031.51 $\pm$ 235.75	a,b,c,d,e
	Gilenya	g	1078.57 $\pm$ 239.71	a,b,c,d,e
Third line treatment	Rituximab	h	945.90 $\pm$ 170.23	a,b,c
	Ocrevus	i	962.94 $\pm$ 206.50	a,b,c

Note: a Negative control, b Untreated MS patients, c Rebif, d Avonex, e Betaferon, f Tysabri, g Gilenya, h Rituximab, i Ocrevus. Letters listed in the Significancy column indicate groups showing statistically significant differences ($p < 0.05$) compared with the referenced group.

Among first-line therapies, Rebif-treated patients (473.59 ± 149.64) showed intermediate IL-18 levels comparable to controls, while other interferon-based treatments (Avonex (819.94 ± 313.88) and Betaferon (891.77 ± 185.44)) were associated with significantly higher cytokine expression. Second-line therapies, including Tysabri (1031.51 ± 235.75) and Gilenya (1078.57 ± 239.71), demonstrated the highest IL-18 concentrations, indicating pronounced immune activation. Patients receiving third-line monoclonal antibody therapies (Rituximab (945.90 ± 170.23) and Ocrevus (962.94 ± 206.50)) also exhibited elevated IL-18 levels, although these were modestly lower than those observed in second-line groups. IL-18 gene expression was upregulated with an increase in treatment lines, showing a positive correlation with disease progression and proving that advanced treatment is correlated with increased inflammation.

This study demonstrated significant differences in serum IL-18 levels among MS patients receiving different disease-modifying therapies, including interferon beta-1a (Rebif and Avonex), interferon beta-1b (Betaferon), fingolimod (Gilenya), natalizumab (Tysabri), ocrelizumab (Ocrevus), and rituximab. IL-18 is a key pro-inflammatory cytokine of the IL-1 family that is primarily produced following activation of the NLRP3 inflammasome. It acts as a potent inducer of interferon- γ production and promotes Th1-mediated immune responses [8]. These pathways are strongly implicated in MS immunopathogenesis, where both innate and adaptive immune mechanisms contribute to cen-

tral nervous system inflammation. The elevated IL-18 levels observed in untreated patients compared with healthy controls are consistent with enhanced inflammatory activity and further support the role of IL-18 in MS pathogenesis [6], [7], [16]. Notably, although all treatment groups exhibited altered IL-18 levels, significant differences from healthy controls persisted, suggesting that disease-modifying therapies may not completely normalize IL-18 expression but rather modulate the underlying immune response [17], [18], [19]. Furthermore, the significant differences between untreated and treated patients indicate that these therapies exert measurable immunomodulatory effects on cytokine profiles, including IL-18 [20]. Among treatments, the relatively lower IL-18 levels observed with Rebif compared to other therapies point to a distinct immunomodulatory effect, whereas the elevated levels associated with interferon-based and higher-efficiency therapies suggest differential activation or redistribution of immunopathways. The lack of consistent significant differences among high-efficiency treatments may reflect convergent mechanisms of immune reprogramming despite distant modes of action [21].

Collectively, these findings support the concept that IL-18 is a dynamic biomarker capturing both inflammatory burdens and therapy-specific immune alterations in MS. Unexpectedly, interferon beta-treated patients (Betaferon and Avonex) exhibited higher IL-18 levels than untreated individuals, highlighting the complex and non-suppressive nature of interferon signaling in MS [18], [22]. Rather than uniformly dampening inflammation, interferon beta may enhance antigen presentation and reshape cytokine networks, potentially promoting selective upregulation of inflammasome-associated mediators such as IL-18 [18]. These findings support the emerging view that disease-modifying therapies act by reprogramming the immune response rather than simply suppressing inflammatory activity. The highest IL-18 levels were observed in patients receiving high-efficacy therapies, including Tysabri (natalizumab) and Gilenya (fingolimod), which primarily act by altering lymphocyte trafficking. Despite their clinical effectiveness, these agents induce peripheral immune redistribution, which may underlie the observed cytokine changes. Elevated IL-18 in this setting likely reflects compensatory activation of innate immune pathways or peripheral accumulation of cytokines resulting from restricted immune cell migration into the central nervous system, reinforcing the concept rather than eliminating inflammatory signaling. Patients receiving B cell-depleting therapies, including Rituximab and Ocrelizumab, also exhibited elevated IL-18 levels, suggesting that B-cell depletion may disrupt regulatory cytokine networks and promote compensatory activation of innate immune pathways, including inflammasome signaling. This supports the concept that MS therapies induce immune reprogramming rather than simple immunosuppression [12], [23], [24]. In contrast, lower IL-18 levels in patients treated with Rebif indicate heterogeneity within interferon-based therapies, potentially reflecting differences in dosing, formulation, and pharmacokinetics that shape distinct immunological outcomes [21].

In general, these findings identify IL-18 as a dynamic biomarker that integrates disease-associated inflammation with therapy-induced immune remodeling, capturing the balance between immune suppression, redistribution, and compensatory activation, in line with emerging systems-level perspectives on MS immunopathology. Based on Spearman's correlation analysis in the untreated patient group, EDSS scores showed significant positive correlations with disease activity ($r = 0.66$, $p < 0.01$), disease severity ($r = 0.60$, $p < 0.05$), and the number of relapses ($r = 0.54$, $p < 0.05$). These findings suggest that increased inflammatory activity and recurrent relapses are associated with greater neurological impairment and disease burden in patients with multiple sclerosis. In contrast, serum IL-18 levels did not show significant correlations with EDSS scores, disease activity, disease severity, or the number of relapses. Although IL-18 is recognized as a pro-inflammatory cytokine involved in the activation of Th1 immune responses and the production of interferon- γ , its circulating levels may not directly reflect clinical manifestations at a single time point. The biological activity of IL-18 may be influenced by several factors, including disease stage, immune regulation, temporal fluctuations in inflammatory responses, and individual variability among patients [6], [9]. Moreover, the absence of a significant correlation may be attributed to the relatively limited sample size and the cross-sectional design of the study, as shown in Figure 1, which may reduce the ability to detect subtle associations. Therefore, larger longitudinal studies are needed to further elucidate the relationship between IL-18 expression and clinical outcomes in multiple sclerosis.

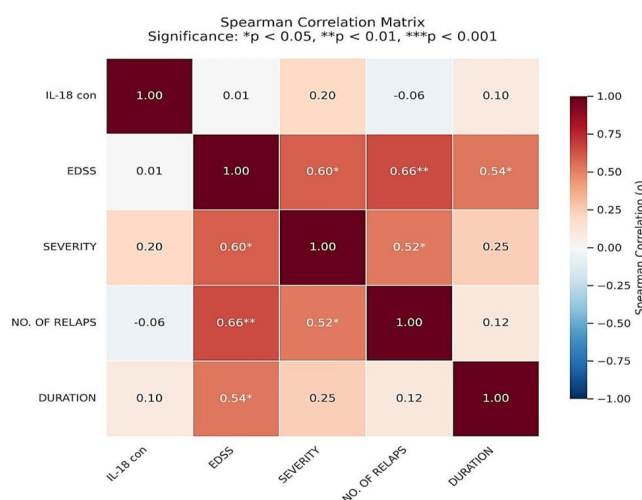


Figure 1. Correlation between IL-18 and clinical factors

ROC curve analysis was performed to evaluate the diagnostic performance of serum IL-18 levels in distinguishing patients with MS from healthy controls, as shown in Figure 2. The analysis yielded an AUC of 0.692 (SE = 0.091, $p = 0.03$), indicating a moderate discriminatory capacity of serum IL-18 for differentiating between the two groups. Although the observed diagnostic accuracy was statistically significant, the AUC value suggests that IL-18 alone may have limited utility as an independent diagnostic biomarker. Nevertheless, these findings support a potential role for IL-18 as a complementary biomarker that could contribute to diagnostic assessment when used in combination with other clinical and laboratory parameters.

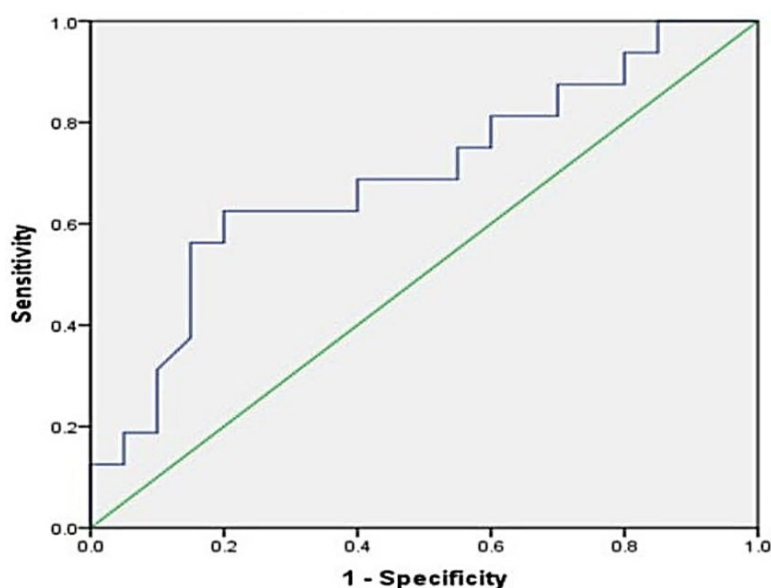


Figure 2. ROC analysis of IL-18 in the untreated patients' group

As this cytokine is a pleiotropic pro-inflammatory that is involved in the immune interaction between innate and adaptive immunity, especially by stimulating the interferon gamma production and amplifying helper T cell responses [25], [26]. In multiple sclerosis, interleukin 18 is associated with the activation of inflammasome activation and neuroinflammation (however, its expression is not specific to the disease) and overlaps with other inflammatory conditions [7], [8]. It also showed variation in the accuracy of the estimate, as the confidence interval ranged between 0.514 and 0.78, as shown in Table 3. The curve also showed a gradual increase above the diagonal reference line, which confirms that the performance of this interleukin is better than chance in distinguishing between MS patients and healthy individuals, although the distinction is not strong.

Table 3. ROC analysis of IL-18 in the untreated patients group

Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.692	0.091	0.050	0.514	0.870

Note: ^a Under the nonparametric assumption, ^b Null hypothesis: true area = 0.5

From the applied perspective, these results oppose its use as a standalone diagnostic tool, but they support its inclusion in the group of biomarkers for this disease. It is recommended to investigate the molecular pathways that underlie the influence of certain treatments on the cytokine levels. This paper has several weaknesses, such as a small number of samples, the cross-sectional research design, and different durations of treatment for the patients. These factors may affect generalizability and causal inference of the findings.

CONCLUSION

The present study demonstrated significantly elevated serum IL-18 levels in patients with MS compared with healthy controls. Furthermore, IL-18 concentrations varied according to treatment status, with untreated patients exhibiting higher levels than those receiving disease-modifying therapies, suggesting a potential association between IL-18 expression and inflammatory activity. Despite these findings, no significant correlations were observed between serum IL-18 levels and clinical parameters, including EDSS score, disease severity, disease duration, and relapse frequency. In addition, ROC curve analysis indicated only moderate diagnostic performance, suggesting that IL-18 alone is unlikely to serve as a reliable standalone diagnostic biomarker. Collectively, these results support a potential role for IL-18 as an immunological marker reflecting inflammatory processes in multiple sclerosis. However, its clinical utility may be enhanced when combined with other biomarkers and clinical indicators. Further large-scale longitudinal studies are required to clarify its role in disease monitoring, prognosis, and therapeutic response.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Zahraa Mazin: Data curation, Formal statistical analysis, Visualization, Writing – original draft. Alia Essam Mahmood: Methodology, writing the manuscript, Project administration, Writing – review & editing. Gheyath Abd Ali Shallal: Investigation, Supervision.

FUNDING

This research received no external funding.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Biology, College of Science, Mustansiriyah University, for support in the present work.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

The study protocol received ethical approval from the ethics committee at Mustansiriyah University/ college of Science (Ref.: BCSMU/1025/0012B), and the written informed consent of each participant and blood sample donor was obtained.

DECLARATION OF GENERATIVE AI USE

During the preparation of this manuscript, the authors used Grammarly for grammar checking and language polishing. After using this tool, the authors thoroughly reviewed and revised the generated content, taking full responsibility for the accuracy and integrity of the final manuscript.

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