Research Article

Al-Iraqia Medical College Journal (AIMCJ)
ISSN (Online): 3104-4565
ISSN (Print): 3104-4557

ARTICLE INFO
Received: 17/11 / 2025
Revised: 5/ 1/ 2026
Accepted: 6/ 1/ 2026
Publish online: 15 /4 / 2026

*Corresponding Author: Worood Younis Azawi
Email: woroodyounis@mtu.edu.iq

CITATION
Worood Younis Azawi. Association of IL-6 and TNF-α with IgA/IgG in Iraqi Patients with Multiple Sclerosis. *AIMCJ*. 2026;3(1): 14-28.

DOI: https://doi.org/10.58564/AIMCJ3.1.2026.242

Association of IL-6 and TNF-α with IgA/IgG in Iraqi Patients with Multiple Sclerosis

Worood Younis Azawi [1*]

[1] Balad Technical Institute, Middle Technical University, Salah Al-Din, Iraq

Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating central nervous system disorder with an immune-mediated demyelination.

Proinflammatory cytokines, including IL-6 and TNF-α, have a crucial role in the induction of neuroinflammation, and immunoglobulin dysfunction (IgA and IgG) is evidence of humoral immune reactivity. In Iraqi patients, the relationship between these markers remains poorly recognized. The study aims to assess the serum IL-6, TNF-α, IgA, and IgG levels in Iraqi patients with MS and examine their interrelationships. Clinically diagnosed patients with MS and 60 age and sex-matched healthy controls, aged 18–60 years, were included in the case-control study. Serum IL-6 and TNF-α levels were measured by enzyme-linked immunosorbent assay (ELISA), and IgA and IgG levels were measured by the method of nephelometry. Statistical analysis was performed using t-tests and Pearson's correlation, with $p < 0.05$ considered statistically significant.

The serum levels of IL-6 and TNF-α were significantly higher for patients with MS (15.87 ± 4.62 pg/mL; 24.65 ± 6.73 pg/mL) than for controls (7.94 ± 2.18 pg/mL; 12.18 ± 3.54 pg/mL, respectively; $p < 0.001$). Levels of IgG (14.52 ± 2.96 g/L) and IgA (3.01 ± 0.78 g/L) were also significantly elevated in patients than in controls (11.84 ± 2.41 g/L and 2.35 ± 0.65 g/L, $p < 0.01$). A significant positive correlation between IL-6 and IgG ($r = 0.418$, $p< 0.001$) and also TNF-α and IgA ($r = 0.496$, $p < 0.001$) was found.

A strong association exists between cytokines and immunoglobulins, and concurrent elevations of IL-6, TNF-α, IgA, and IgG are observed in Iraqi MS patients. The strong correlation between TNF-α and IgA might be the result of distinct genetic or environmental factors, highlighting the necessity of region-specific biomarker research and customized monitoring plans.

Keywords: Multiple sclerosis, cytokines, IL-6, TNF-α, IgA, IgG, Iraqi population.

AIMCJ. (2026),3; (1):14-28
https://doi.org/10.58564/AIMCJ3.1.2026.242

Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system (CNS) featuring inflammatory demyelination, axonal pathology, and gliosis (1,2). Etiology of MS is multifactorial and includes genetic susceptibility, the environment, and disruption of both innate and adaptive immune pathways (3). From an immunopathological perspective, MS lesions are characterized by the presence of activated T lymphocytes, macrophages, and B cells, which result in myelin injury and neuronal loss (4).

Among the proinflammatory mediators associated with MS, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are prominent cytokines that mediate immune cell activation and sustain CNS inflammation. IL-6 is a pleiotropic cytokine synthesized by monocytes, astrocytes, microglia, and B cells; it is involved in B-cell maturation into plasma cells and in the synthesis of immunoglobulins, in particular IgG and IgA (5). Increased IL-6 titers are detected in serum and cerebrospinal fluid (CSF) in MS and are linked to disease activity, relapses, and disability progression (6,7).

TNF-α, primarily secreted by activated macrophages, microglia, and T cells, plays a dual role in MS by acting as a pro-inflammatory cytokine by upregulating adhesion molecules and promoting leukocyte migration to the CNS, and as a neuroprotective cytokine through TNF receptor 2-mediated pathways (8). Increased TNF-α has been associated with exacerbations of MS and MRI lesion activity (9). Nevertheless, clinical trials involving treatment with TNF-α inhibitors have exacerbated MS, highlighting its intricate function in disease development (10).

Immunoglobulins serve as a signature of humoral immune activation in MS. IgG is the most abundant immunoglobulin in the CSF-derived oligoclonal bands, present in over 90% of individuals with MS and representing a major diagnostic biomarker (11). IgA, less commonly investigated in the MS setting, is primarily associated with mucosal immune function, with a possible reflection of gut–CNS immune crosstalk, an emerging field in neuroimmunology (12). IL-6 promotes both IgA and IgG production, and TNF-α indirectly affects antibody production by inducing inflammation (13). Significant immunogenetic and environmental differences require population-specific research, even though the roles of IL-6, TNF-α, and immunoglobulins in MS pathophysiology have been described in Western and East Asian populations (14,15). Due to their high prevalence of vitamin D deficiency, different genetic backgrounds in HLA and cytokine-related alleles, and endemic exposures to particular mucosal pathogens, the Iraqi population offers a unique context (14,16). These elements may cause a different immunopathological profile in MS and are known to significantly alter systemic immune responses (17,18). Thus, this study is a critical examination of a unique immunophenotype rather than just a replication. It is the first thorough examination of how these important proinflammatory cytokines and immunoglobulins interact with one another in Iraqi MS patients. Clarifying this profile is crucial because it could identify pathogenic drivers unique to a population, like an increased gut-brain axis interaction, and it is a requirement for creating pertinent diagnostic and treatment plans that are adapted to the disease's local context.

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Objectives

1. To estimate the levels of serum IL-6, TNF-α, IgA, and IgG in Iraqi multiple sclerosis (MS) patients in comparison to those in a control group.
2. The relationships of IL-6 and TNF-α levels with serum Ig concentrations (IgA, IgG) were also determined.
3. To evaluate relationships of the immunologic markers with clinical features (such as type of MS, duration of disease, and the Expanded Disability Status Scale "EDSS scores").

Materials &Methods

Study Design

This case–control study was conducted at the Department of Neurology, Middle East Hospital, Baghdad, Iraq, during the period January to May 2025. The study was approved by the Intervention Ethics Committee of the Middle Technical University, Iraq. Participants all signed informed consent for study enrollment.
A total of 120 individuals were recruited, including 60 patients diagnosed with multiple sclerosis according to the 2017 revised McDonald criteria (10), aged 18–60 years, with 38 males and 22 females. The control group consisted of 60 age- and sex-matched healthy individuals, comprising 42 males and 18 females, selected from healthy blood donors and hospital staff at Middle East Hospital, Baghdad, Iraq.

Sample Size Justification

The sample size was calculated from pilot data for a similar cytokine and isotypes study in MS patients showing moderate to large responses in serum IL-6 and TNF-α distinction between MS patients and healthy subjects (effect sizes between 0.6 to 0.9; Cohen's d). By means of G*Power 3.1 software, a two-tailed independent samples t-test with $\alpha = 0.05$ and power $(1 - \beta) = 0.90$, a minimum of 52 subjects for each group was necessary to detect a medium effect size ($d = 0.65$). For possible dropouts and variance of the patients, we increased the sample size to 60 patients and 60 controls with the corresponding power to find clinically significant differences in cytokine and immunoglobulin levels >90%.

Inclusion Criteria

MS group:

- Confirmed diagnosis of MS based on clinical findings by a neurologist at the Department of Neurology, Middle East Hospital, Baghdad, Iraq
- Age 18–60 years.
- Disease duration ≥ 6 months.
- No recurrence within 4 weeks before blood sampling.

Control group:

- Age-matched healthy controls.
- No previous neurologic or autoimmune disease.

Exclusion Criteria

- Recent infection (acute or vaccination during the last 1 month).
- Treatment with systemic corticosteroid or immunosuppressants within 4 weeks before sampling.
- Autoimmune, chronic inflammatory, or malignant diseases.

Control for Potential Confounders

While subjects with recent infections, autoimmune diseases, or receiving recent immunosuppressive therapy were excluded, other confounders, such as vitamin D levels, nutritional habits, or history of latent infections, were not directly assessed in our study. These are recognized to affect the systemic level of cytokines and immunoglobulins. The inclusion and

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exclusion criteria were intended to limit major inflammatory influences, but we cannot exclude residual confounding. Laboratory measurement of vitamin D and latent infection (e.g., EBV, CMV) status, and dietary assessment, should be performed in future studies, in order to adjust statistically for these factors.

Clinical Evaluation

In MS subjects, demographic and clinical information were collected, including disease subtype (Relapsing-Remitting RRMS, Secondary Progressive SPMS, or Primary Progressive PPMS), disease duration, and ongoing treatment. Disability was assessed by using EDSS in the Department of Neurology, Middle East Hospital, Baghdad.

Sample Collection and Processing

Venous blood (5 ml) was drawn from each subject between 8 and 10 am, following an overnight fast. Blood samples were obtained from fresh, in serum separator tubes, and then stored at room temperature, clotted, and centrifuged at 3,000rpm for 10 minutes. Serum samples were aliquoted and stored at –80 °C until analysis.

Cytokines Measurement (IL-6 and TNF-α)

Serum IL-6 and TNF-α levels were measured by commercial ELISA kits (e.g., R&D Systems, MN, USA) following the manufacturer's instructions. Absorbance at 450 nm was measured with a microplate reader (BioTek Instruments), and the values were converted to concentrations using standard curves.

Immunoglobulins (IgA and IgG) measurement

Serum levels of IgA and IgG were determined by nephelometry (Siemens BN™ II System, Siemens Healthcare Diagnostics, Marburg, Germany). Values were presented in g/L, and all experiments were conducted in duplicates.

Statistical Analysis

Statistical analysis was conducted using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Normal distribution of the data was evaluated by the Shapiro–Wilk test. Continuous variables were indicated with mean ± SD or median (interquartile range), and categorical variables using numbers and percentages. Between-group comparisons were made by an independent-samples t-test or Mann–Whitney U test when applicable. Categorical variables were compared with the chi-square test. Pearson's or Spearman's correlation coefficients were applied to examine relationships among cytokines, immunoglobulins, and EDSS scores. $P < 0.05$ was considered to be statistically significant.

Results:

A summary of the 120 subjects recruited is presented in Table 1, consisting of 60 MS patients (38 males, 22 females) and 60 healthy controls (42 males, 18 females). There was no statistical difference between the mean age of both groups ($p = 0.472$). Regarding MS patients (73.3%) had RRMS, (16,7%) SPMS, and (10.0%) PPMS. Mean disease duration was 8.21 ± 4.17 years, mean EDSS score was 3.42 ± 1.28.

Table 2 showed that the mean serum levels of IL-6 of MS subjects were significantly higher than those of controls ($p < 0.001$). Mean TNF-α levels were also higher in the MS group than in the control group ($p < 0.001$). The cytokine differences showed very large effect sizes (Cohen's $d > 1.9$), suggesting a significant biological effect that went beyond statistical significance.

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Table 1: The study participants' clinical and demographic details.

Variable	MS patients (n=60)	Controls (n=60)	*p*-value
Age (years), mean ± SD	37.85 ± 10.12	36.72 ± 9.86	0.472
Gender (M/F)	38 / 22	42 / 18	0.439
Disease duration (years)	8.21 ± 4.17	—	—
EDSS score, mean ± SD	3.42 ± 1.28	—	—
RRMS / SPMS / PPMS	44 / 10 / 6	—	—

Relapsing-Remitting RRMS, Secondary Pro-aggressive SPMS, or Primary Progressive PPMS,
"Values of p<0.05 were considered to be significant."

Table 2: Serum cytokine levels in healthy controls and MS patients.

Cytokine	MS patients (mean ± SD)	Controls (mean ± SD)	*p*-value	Effect Size (Cohen's d) (95% CI)
IL-6 (pg/mL)	15.87 ± 4.62	7.94 ± 2.18	<0.001	1.98 (1.48 to 2.47)
TNF-α (pg/mL)	24.65 ± 6.73	12.18 ± 3.54	<0.001	2.06 (1.55 to 2.56)

"Values of p<0.05 were considered to be significant."

Table 3. Serum immunoglobulin levels in healthy controls and MS patients.

Immunoglobulin	MS patients (mean ± SD)	Controls (mean ± SD)	*p*-value	Effect Size (Cohen's d) (95% CI)
IgA (g/L)	3.01 ± 0.78	2.35 ± 0.65	<0.001	0.83 (0.43 to 1.23)
IgG (g/L)	14.52 ± 2.96	11.84 ± 2.41	0.002	0.87 (0.46 to 1.27)

"Values of p<0.05 were considered to be significant."

Table 4 shows the correlation coefficients for immunoglobulins, cytokines, and EDSS in MS patients.

Variable	IgA (*r*, *p*)	IgG (*r*, *p*)	EDSS (*r*, *p*)	(95% CI) for *r* with IgA
IL-6	0.542, <0.001	0.418, 0.001	0.451, <0.001	0.33 to 0.70
TNF-α	0.496, <0.001	0.387, 0.002	0.428, 0.001	0.28 to 0.66

"Values of p<0.05 were considered to be significant.

Table 3 revealed that serum IgA and IgG levels were markedly increased in MS patients when compared with the control group. The differences for both immunoglobulins were statistically significant ($p < 0.001$ for IgA; $p = 0.002$ for IgG).

The immunoglobulin differences showed very large effect sizes (Cohen's $d > 0.8$), suggesting a significant biological effect that went beyond statistical significance.

Table 4 showed that in patients with MS, IL-6 levels significantly correlated with IgA ($r = 0.542$, $p < 0.001$) and IgG ($r = 0.418$, $p = 0.001$). TNF-α was also positively associated with IgA ($r = 0.496$, $p < 0.001$) and IgG ($r = 0.387$, $p = 0.002$).

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Additionally, there were significant moderate positive correlations between IL-6 and TNF-α and EDSS scores ($r = 0.451$, $p < 0.001$, and $r = 0.428$, $p = 0.001$, respectively). TNF-α and IgA had a strong correlation ($r = 0.496$, 95% CI [0.28 to 0.66], $p < 0.001$), suggesting a significant and dependable association.

According to the previous results, serum levels of IL-6, TNF-α, IgA, and IgG were significantly higher in MS patients compared to healthy controls. The significant associations between cytokines and immunoglobulins, as well as between cytokines and EDSS scores, are visually indicated in Figures 1, 2. This suggests that both IL-6 and TNF-α may contribute to the humoral immune response and disease severity in Iraqi MS patients.

Multiple linear regression analyses were further conducted to explain the independent association of the immunological markers with clinical determinants (Tables 5 and 6). After correction for age, sex, disease duration, and MS subtype in a stepwise multiple regression analysis, serum IL-6 levels were independently driven by disease duration ($p = 0.008$), IgG levels ($p = 0.015$), and degree of disability (EDSS score; $p = 0.038$), with a total variance accounted for of 36% for IL-6 ($p < 0.001$).

In contrast, higher IgA ($p < 0.001$), longer disease duration ($p = 0.006$), and a progressive disease course ($p = 0.042$) were independent predictors of increased TNF-α, which explained 42% of the variance ($p < 0.001$). Last, an EDSS clinical disability regression model showed that increased EDSS was independently associated with increased IL-6 ($p = 0.004$), increased TNF-alpha ($p < 0.012$), and longer disease duration ($p = 0.021$), with 38% of the variance in disability explained ($p < 0.001$). These analyses show that the correlations found are strong and highlight these cytokines and immunoglobulins as mutually predictive of disability independent of important demographic and clinical covariates.

Moreover, IgA level was the strongest independent predictor, according to the TNF-α regression model (95% CI [0.20 to 0.62], $p < 0.001$), with the CI not crossing zero, suggesting a strong positive association

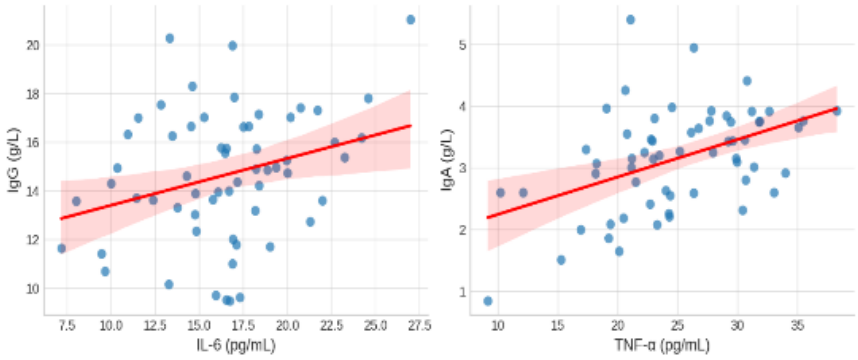

Figure 1. Scatterplots of correlation with regression lines and 95% confidence intervals for IL-6 vs. IgG and TNF-α vs. IgA.

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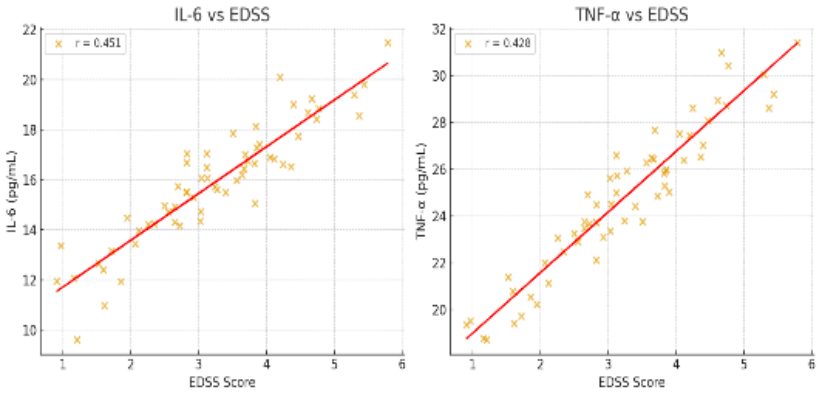

Figure 2 illustrates the positive correlations between EDSS scores and serum IL-6 and TNF-α concentrations.

When compared based on clinical subtype, serum IL-6 and TNF-α levels were the highest in SPMS patients , followed by PPMS and RRMS. A trend similar to that found for the cytokine levels was evident for IgA and IgG, which were also highest in SPMS subjects. Univariate analysis of one-way ANOVA further showed that there were significant differences among the subtypes for IL-6 ($p = 0.038$) and TNF-α ($p = 0.042$); IgA and IgG were not found to do so, as shown in Table 7.

The duration of the disease was divided into 10 years. High levels of both IL-6 and TNF-α were associated with higher disease duration (more than 10 years in both high vs. <5 years low, IL-6: $p = 0.022$; TNF-α: $p = 0.018$). By contrast, the longer the duration of disease, the higher the IgA levels ($p = 0.041$); there was no such correlation for IgG ($p = 0.089$).

Based on previous results, as shown in Figure 3, and consistent with the pattern of increasing disease severity, the concentrations of TNF-α and IL-6 were lowest in RRMS, intermediate in PPMS, and highest in SPMS

Table 5: Multiple Linear Regression Analysis for Serum IL-6 and TNF-α Level Predictors

Dependent Variable	Independent Variable	β Coefficient	95% Confidence Interval	p-value
IL-6 (pg/mL)	Disease Duration	0.32	0.09 to 0.55	0.008
	IgG (g/L)	0.28	0.06 to 0.50	0.015
	EDSS Score	0.24	0.02 to 0.46	0.038
	Model Summary	**Adj. $R^2 = 0.36$**		**<0.001**
TNF-α (pg/mL)	IgA (g/L)	0.41	0.20 to 0.62	<0.001
	Disease Duration	0.29	0.09 to 0.49	0.006
	MS Subtype (Progressive)	0.22	0.01 to 0.43	0.042
	Model Summary	**Adj. $R^2 = 0.42$**		**<0.001**

β: Standardized beta coefficient.

"Values of p<0.05 were considered to be significant."

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Table 6: EDSS Score Predictors of Clinical Disability Using Multiple Linear Regression Analysis

Dependent Variable	Independent Variable	β Coefficient	95% Confidence Interval	p-value
EDSS Score				
	IL-6 (pg/mL)	0.31	0.11 to 0.51	0.004
	TNF-α (pg/mL)	0.27	0.06 to 0.48	0.012
	Disease Duration	0.25	0.04 to 0.46	0.021
	Model Summary	**Adj. R^2 = 0.38**		**<0.001**

"Values of p<0.05 were considered to be significant."

Table 7. Cytokine and immunoglobulin serum levels depending on MS subtype and duration of disease

Variable	RRMS (n=44) Mean ± SD	SPMS (n=10) Mean ± SD	PPMS (n=6) Mean ± SD	*p*-value (Subtype)	<5 years (n=20) Mean ± SD	5–10 years (n=25) Mean ± SD	>10 years (n=15) Mean ± SD	*p*-value (Duration)
IL-6 (pg/mL)	15.21 ± 4.57	17.45 ± 4.18	16.88 ± 4.26	0.038	14.92 ± 4.12	16.08 ± 4.55	17.69 ± 4.23	0.022
TNF-α (pg/mL)	23.98 ± 6.41	26.92 ± 5.11	25.34 ± 5.64	0.042	23.11 ± 6.05	24.87 ± 6.34	27.01 ± 5.78	0.018
IgA (g/L)	2.97 ± 0.76	3.15 ± 0.82	3.09 ± 0.79	0.218	2.85 ± 0.70	3.02 ± 0.79	3.21 ± 0.81	0.041
IgG (g/L)	14.41 ± 2.94	14.88 ± 3.02	14.67 ± 2.91	0.463	14.26 ± 2.85	14.49 ± 2.98	14.91 ± 3.04	0.089

"Values of p<0.05 were considered to be significant."

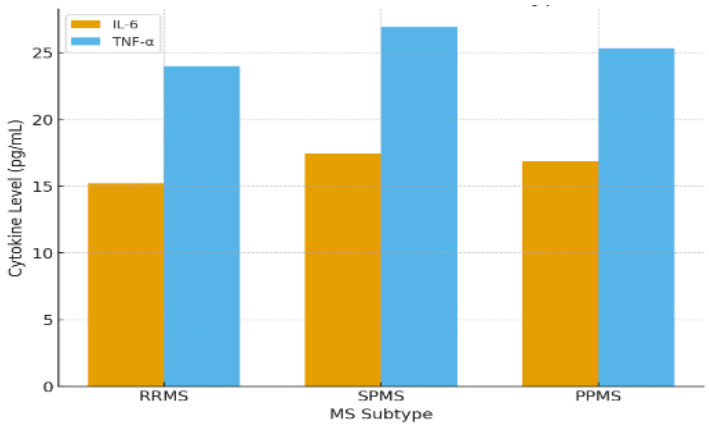

Figure 3. Showing comparison of IL-6 and TNF-α levels across RRMS, SPMS, and PPMS.

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Discussion

In this study, the serum levels of Iraqi MS patients have concurrently elevated levels of immunoglobulins (IgA, IgG) and proinflammatory cytokines (IL-6, TNF-α), with significant positive correlations found between these immune markers and clinical disability as assessed by the EDSS. The strength and pattern of these associations, especially the strong TNF-α/IgA axis and the highest levels in progressive subtypes (SPMS/PPMS), suggest a potentially unique immunopathological signature in the Iraqi population that merits mechanistic interpretation, even though these findings are consistent with the mainstream paradigm of MS as a disease of combined innate and adaptive immune dysregulation.

This study offers the first description of a simultaneous increase in immunoglobulins (IgA, IgG) and proinflammatory cytokines (IL-6, TNF-α) in the serum of Iraqi MS patients, demonstrating strong associations between the two and with clinical disability. These findings are novel and significant because they show a potentially distinct immunopathological signature within this particular population, going beyond the validation of a general inflammatory state. These observations are in line with the worldwide view that MS is a disease of both innate and adaptive immunity (18,19), but the strength and profile of these associations, and particularly the strong correlation between TNF-α and IgA, may reflect genetic and environmental factors that could bias the immune response in unique ways.

Multivariate regression analyses, which accounted for potential confounders such as age, gender, duration of disease, and MS subtype, further confirmed the strong positive correlations between IL-6, TNF-α, immunoglobulins, and disability. According to these analyses, TNF-α levels were independently linked to IgA levels and a progressive disease phenotype, whereas IL-6 levels were independently predicted by IgG levels and disability (EDSS score). Importantly, in addition to disease duration, both cytokines were found to be independent predictors of neurological disability (EDSS). The conclusion that the observed associations are not just epiphenomena of broader disease activity or duration is supported by these regression models. Rather, they imply that these particular immune mediators have a more direct, possibly contributing role in the humoral response and the development of the disease in Iraqi MS patients. The hypothesis of a unique immunopathological axis in this population is supported by the strength of the independent association between TNF-α and IgA ($\beta = 0.41$, $p < 0.001$), which is likely shaped by the particular genetic and environmental context of Iraq, such as endemic mucosal pathogens or specific HLA alleles influencing cytokine production.

Additionally, the discovery that both cytokines independently contribute to the EDSS score highlights their function as potential causes of cumulative neurological damage in addition to being indicators of inflammation. This strengthens the case for their study as therapeutic targets, especially for progressive forms of the illness where there are few available treatments.

To our knowledge, this is the first study that characterizes the combined serum cytokine-immunoglobulin profile in Iraqi MS patients. Although the high levels of these markers have been reported in other populations, the magnitude and the pattern of the changes reported

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here, especially for the rise in the levels of both IgA/IgG, along with proinflammatory cytokines, might be attributed to the synergistic effect that may arise between the specific environmental and genetic factors that are specific for that region. The high prevalence of vitamin D deficiency, unique infectious exposures, and potential genetic susceptibilities in HLA and cytokine-related alleles in Iraq may differentially influence immune activation compared to Western or East Asian populations. The increased association we found between TNF-α and IgA in this cohort when compared with previous studies may indicate a greater influence of the mucosal and systemic immune compartment in this population that can be related to endemic microbial exposures and gut–CNS immune interactions. These observations not only address the existing gap in Middle Eastern MS immunology but also highlight the importance of population-specific biomarker studies for guiding personalized monitoring and therapeutic interventions.

Firstly, the circulating levels of IL-6 and TNF-α were higher in patients with MS compared to their healthy counterparts, as shown in Table 2. This observation suggests a state of increased systemic inflammation in MS, aligning with the known pathophysiology characterized by chronic immune-mediated demyelination and neurodegeneration (20). Similar increased levels of IL-6 and TNF-α in MS cases were also identified in previous studies (14)(22). Therefore, these findings underscore the involvement of proinflammatory cytokines in the induction and maintenance of CNS inflammation.

Several factors may influence the observed cytokine elevation in our study:

- Genetic susceptibility: Some of the HLA alleles, which are more frequent in Middle Eastern populations, and polymorphisms of cytokine genes could raise basal cytokine production (16).
- Environmental factors: Viral infections, urban pollution, and vitamin D deficiency as an endemic characteristic in Iraq may induce upregulation of systemic inflammatory mediators (14,22).
- Stage of disease: Patients had not been treated with immunosuppression shortly before the study; however, the confounding suppression of cytokine levels is minimized.

Taken together, these mechanisms may contribute to the noted increase in IL-6 and TNF-α, but also suggest them as candidate biomarkers of immune activation in MS.

In addition to the cytokine findings, as shown in Table 3, the study showed significantly higher levels of IgG and IgA in MS than in controls ($p < 0.01$). In short, the findings indicate an augmented humoral immune response, likely secondary to cytokine-mediated B-cell activation.

Specifically, IL-6 is reported to facilitate B-cell differentiation into plasma cells and to enhance antibody production (23). Accordingly, the positive relationships between IL-6/TNF-α to IgA/IgG reported in this study lend support to such relationships as shown in Table 4 ($p < 0.01$), indicating that systemic inflammation leads directly to humoral immune activation in MS.

Therefore, the positive association of IL-6 and IgG ($r = 0.418$, $p = 0.001$) is not merely an associative finding but is indicative of a direct pathophysiological driver in MS; IL-6 is the most central factor to drive B cells to differentiate into antibody-producing plasma cells (23). It suggests the presence of an active IL-6-mediated humoral immunity. Increases in serum IgG,

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though smaller than in the CSF, denote systemic B cell stimulation that may parallel and contribute to the well-established intrathecal production of IgG observed in MS; this observation highlights the therapeutic potential of IL-6 signaling pathway-targeted therapies, such as anti-IL-6R antibodies (e.g., tocilizumab) to suppress pathogenic B cell responses in MS supported by recent experimental and early clinical trial work (24).

These observations are in line with the study of Wang, Q., et al., which found a mild IgG serum increase in RRMS patients. Interestingly, changes in IgA are less consistently described (25). Increased levels of IgA might also be related to the mucosal immune system activity, and/or endemically encountered pathogens, as previously mentioned, which applies to the Iraqi population, especially (26).

Serum levels of TNF-α and IL-6 were significantly higher in patients with SPMS and PPMS than in those with RRMS, according to our stratified analysis ($p < 0.05$), as shown in Table 7. The idea that progressive multiple sclerosis is defined by a change from acute, relapse-driven inflammation to a more chronic, smoldering, and compartmentalized inflammatory process within the central nervous system is consistent with this gradation.

There are multiple ways to explain why these cytokines are consistently elevated in progressive disease. Resident-activated microglia and astrocytes in the central nervous system (CNS) are a continuous source of IL-6 and TNF-α, causing oxidative stress, low-grade neuroinflammation, and remyelination failure (1). Both cytokines' associations with higher EDSS scores (IL-6: r=0.451; TNF-α: r=0.428) further substantiate their roles as contributors to cumulative neurological damage and disability. Furthermore, the observation that IgA levels rose with longer disease duration ($p = 0.041$) implies that the humoral immune response, which may be influenced by the chronic cytokine milieu, solidifies over time. This is in line with more recent research that highlights how B cells and plasma cells help form lymphoid-like structures in the meninges of patients with progressive multiple sclerosis. These structures act as local factories for the production of cytokines and antibodies (25). Our findings imply that there is a systemic correlate to this intrathecal process, with serum levels representing the burden of persistent, non-resolving inflammation.

Overall, our IL-6 and TNF-α levels are consistent with what has been reported in previous studies from Eastern and Western populations, a fact that suggests that MS pathobiology may be largely preserved across ethnic groups (15,21).

While Immunoglobulins (IgG) in cerebrospinal fluid (CSF) and oligoclonal bands persist as the classic markers, the slightly elevated serum IgG levels that have been described in individuals with MS, albeit modest, and the associated B-cell and plasma cell activation provide additional systemic confirmation of MS presence (25). This IgA elevation appears to be more pronounced in regions with high microbial exposure and is therefore associated with both environmental and immunological conditions (26).

Increased levels of both cytokines and immunoglobulins in this group suggest a synergistic model in which the proinflammatory cytokines crosstalk to promote B-cell activity, resulting in elevated immunoglobulins and subsequently disease exacerbation.

However, the most interesting discovery in our study, as shown in Table 4, was the high

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correlation between TNF-α and IgA ($r = 0.496$, $p < 0.001$), which is higher than commonly observed in Western cohorts. We speculate that local environmental conditions could modulate this particular immunological signature. The endemicity of some mucosal pathogens in Iraq may cause a status of chronic mucosal immune activation (24), in which TNF-α-induced inflammatory and barrier defense could, in turn, continuously drive the production of IgA. This is particularly relevant given that the gut-brain axis is increasingly being recognized as a crucial pathway in multiple sclerosis (MS) (17). Notably, new research has shown that MS patients' gut-derived IgA+ B cells can migrate to the central nervous system (CNS) (27). Thus, this pre-existing, systemically available pool of TNF-α and pathogen-experienced IgA+ B cells may directly cause or worsen neuroinflammation in people with a genetic predisposition to MS. Given the known global disparities in MS (28), this possible synergy between endemic mucosal challenge and central autoimmune disease merits additional research through focused serological and microbiome studies.

Clinical and Research Implications

Collectively, these findings indicate that IL-6 and TNF-α, as well as serum IgA and IgG, may be simple biomarkers for the systemic activity in the immune system of MS patients, and they underscore the need for population-based studies due to genetic and environmental factors that affect these immunological features. Further studies are needed to assess the variation of these markers over time and their association with disease activity, relapse frequency, and response to treatment.

In summary, this study shows that Iraqi MS patients' serum levels of immunoglobulins (IgA, IgG) and pro-inflammatory cytokines (IL-6, TNF-α) are elevated simultaneously, and there are notable correlations between these biomarkers and clinical disability scores. A key component of MS immunopathology, the strong correlation between IL-6 and IgG highlights the cytokine's critical function in promoting B-cell differentiation and antibody production (23,25). More significantly, a possible population-specific immunopathological axis is suggested by the exceptionally strong correlation between TNF-α and IgA, as well as the fact that patients with progressive disease phenotypes have the highest levels of these markers. We propose that by priming systemic and possibly CNS-compartmentalized humoral immunity through the gut-brain axis, endemic environmental factors like prolonged exposure to mucosal pathogens may be the cause of this (27,28). Although these inflammatory pathways are known to occur in multiple sclerosis worldwide (16,21), the unique pattern seen here highlights the crucial role that regional and genetic factors play in disease mechanisms. Thus, in addition to confirming the role of these important immune mediators in MS, our results suggest that IL-6, TNF-α, and immunoglobulins are readily available biomarkers of disease activity and progression in the Iraqi population, underscoring the need for regionally specific biomarker studies.

Limitations and Future Directions

Although our study does provide important information on the immune profile of Iraqi multiple sclerosis patients, there are several limitations. The absence of correlation between biomarker levels and recent clinical disease activity (such as relapse rate or time since last relapse) is a significant limitation. As a result, we

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are unable to identify whether the elevated levels of TNF-α, IgA, IgG, and IL-6 are specifically associated with acute inflammatory attacks or reflect a chronic state of immune dysregulation. To determine whether these markers change with relapse activity and could be used as predictive tools, future prospective studies with frequent sampling are required. Secondly, although the sample size for statistical analysis was acceptable, larger multicenter populations may aid in generalizing the current results over different Iraqi regions and ethnic groups. Third, only blood-based biomarkers were evaluated, and the addition of cerebrospinal fluid (CSF) measures could provide a more direct representation of CNS immune activity. Fourth, confounders, such as subclinical infections, diet, and Vitamin D status, were not well adjusted, which may have affected the levels of immunoglobulins, especially IgA. Lastly, genetic and environmental risk factor analysis should be combined with serial serum and CSF immunological profiling in future studies. This would assist in determining the predictive value of TNF-α, IgA, IgG, and IL-6 for aggressive disease courses. By showing a quantifiable improvement in the course of the disease, intervention trials that target these cytokines may eventually define their clinical utility.

Conclusion

This study confirms the universal role of TNF-α and IL-6 in MS immunopathology. It identifies a unique immunophenotype in Iraqi patients, characterized by a strong TNF-α/IgA axis and co-elevation of immunoglobulins. This suggests that, in addition to common global mechanisms, the disease's immune signature is strongly influenced by region-specific factors that may act via the gut-brain axis. To monitor disease activity and progression in Iraqi and similar Middle Eastern populations, these easily measurable biomarkers (IL-6, TNF-α, IgA, and IgG) may be especially useful. This highlights the need for regionally specific biomarker research and treatment approaches.

Ethical approval

Ethical approval was obtained from the ethical committee of the Middle Technical University, Iraq (2603, 31/12/2024).

Acknowledgment

The author would like to thank all the participants for their voluntary participation and for providing the data.

Funding: nil

Conflicts of interest: nil

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