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

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Abstract

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

Proinflammatory cytokines, including IL-6 and TNF-α, play a crucial role in neuroinflammation, and immunoglobulin dysfunction (IgA and IgG) reflects humoral immune reactivity. In Iraqi patients, the relationship between these markers remains poorly recognized.

The study aims to assess serum levels of IL-6, TNF-α, IgA, and IgG in Iraqi patients with MS and to examine their interrelationships.

Sixty patients with clinically diagnosed 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 nephelometry. Statistical analyses were performed using t-tests and Pearson's correlation; $p < 0.05$ was considered statistically significant.

The serum levels of IL-6 and TNF-α were significantly higher in patients with MS (15.87 ± 4.62 pg/mL; 24.65 ± 6.73 pg/mL) than in 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 compared with controls (11.84 ± 2.41 g/L and 2.35 ± 0.65 g/L, $p < 0.01$). Significant positive correlations were found between IL-6 and IgG ($r = 0.418$, $p < 0.001$) and between TNF-α and IgA ($r = 0.496$, $p < 0.001$).

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 may reflect distinct genetic or environmental factors, underscoring the need for region-specific biomarker research and tailored monitoring plans.

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

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Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system (CNS) characterized by inflammatory demyelination, axonal pathology, and gliosis (1,2). The etiology of MS is multifactorial, including genetic susceptibility, environmental factors, and disruption of both innate and adaptive immune pathways (3). From an immunopathological perspective, MS lesions are marked by activated T lymphocytes, macrophages, and B cells, which drive 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 produced by monocytes, astrocytes, microglia, and B cells; it promotes B-cell maturation into plasma cells and the synthesis of immunoglobulins, particularly IgG and IgA (5). Increased IL-6 levels are detected in serum and cerebrospinal fluid (CSF) in MS and are associated with 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. It acts 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 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 and may reflect 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 its high prevalence of vitamin D deficiency, diverse HLA and cytokine-related alleles, and endemic exposure to specific mucosal pathogens, the Iraqi population offers a unique context (14,16). These elements may cause a distinct immunopathological profile in MS and are known to alter systemic immune responses (17) significantly. 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 in Iraqi patients with MS. Clarifying this profile is crucial because it can identify pathogenic drivers unique to a population, such as increased gut-brain axis interactions, and is required to create pertinent diagnostic and treatment plans 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 Intervention Ethics Committee of the Middle Technical University in Iraq approved the study. 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 from a similar cytokine and isotype study in MS patients, showing moderate to large responses in serum IL-6 and TNF-α, with a distinction between MS patients and healthy subjects (effect sizes of 0.6-0.9; 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 ($d = 0.65$). To account for potential dropouts and patient heterogeneity, we increased the sample size to 60 patients and 60 controls, with a power of> 90% to detect clinically significant differences in cytokine and immunoglobulin levels.

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

Although subjects with recent infections, autoimmune diseases, or recent immunosuppressive therapy were excluded, other confounders, such as vitamin D levels, nutritional habits, or a history of latent infections, were not directly

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assessed in our study. These are recognized to affect the systemic level of cytokines and immunoglobulins. The inclusion and exclusion criteria were intended to limit major sources of inflammation, but we cannot rule out residual confounding.

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 using the EDSS in the Department of Neurology at 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 blood, placed in serum separator tubes, 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 using commercial ELISA kits (e.g., R&D Systems, MN, USA) according to 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

Levels of serum IgA and IgG were determined by nephelometry using the 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). Data normality was evaluated using the Shapiro–Wilk test. Continuous variables were reported as mean ± SD or median (interquartile range), and categorical variables were reported as numbers and percentages. An independent-samples t-test or Mann made between-group comparisons–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:

All 120 subjects recruited are 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 in age among the groups ($p = 0.472$). Among MS patients, 44 (73.3%) had RRMS, 10 (16.7%) had SPMS, and 6 (10.0%) had PPMS. Mean disease duration was 8.21 ± 4.17 years, and the mean EDSS score was 3.42 ± 1.28.

Table 2 showed that serum IL-6 and TNF-α levels were significantly higher in MS subjects than in controls ($p < 0.001$). 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 ($p < 0.001$ for IgA; $p = 0.002$ for IgG). The Ig 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 ($r = 0.387$, $p = 0.002$). Additionally, there were significant moderate positive correlations between IL-6 and

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TNF-α, and between IL-6 and EDSS scores (r = 0.451, p < 0.001; 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 previous results, serum levels of IL-6, TNF-α, IgA, and IgG were significantly higher in MS patients than in healthy controls. The significant associations between cytokines and immunoglobulins, as well as between cytokines and EDSS scores, are visually indicated in Figures 1 and 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 (β = 0.32, p = 0.008), IgG levels (β = 0.28, p = 0.015), and degree of disability (EDSS score; β = 0.24, p = 0.038), with a total variance accounted for of 36% for IL-6 (p < 0.001).

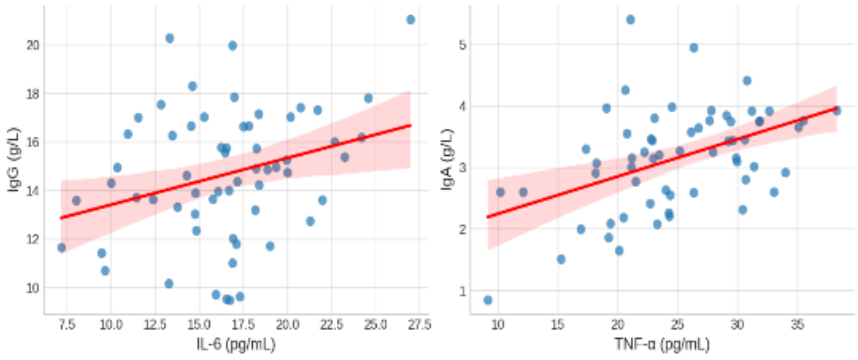

Figure 1. Scatterplots of correlation with regression lines and 95% confidence intervals for IL-6 vs. IgG and TNF-α vs. IgA.

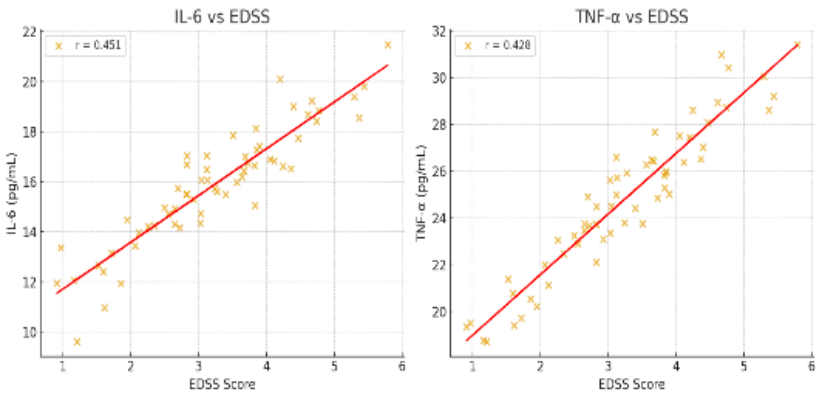

Figure 2 illustrates the positive correlations between EDSS scores and serum IL-6 and TNF-α concentrations.

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In contrast, higher IgA (β = 0.41, p < 0.001), longer disease duration (β = 0.29, p = 0.006), and a progressive disease course (β = 0.22, 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 (β = 0.31, p = 0.004), increased TNF-alpha (β = 0.27, p < 0.012), and longer disease duration (β = 0.25, 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 in the TNF-α regression model (β = 0.41, 95% CI [0.20 to 0.62], p < 0.001), with the CI not crossing zero, suggesting a strong positive association.

When compared based on clinical subtype, serum IL-6 and TNF-α levels were the highest in SPMS patients (IL-6: 17.45 ± 4.18 pg/mL; TNF-α: 26.92 ± 5.11 pg/mL), followed by PPMS (IL-6: 16.88 ± 4.26 pg/mL; TNF-α: 25.34 ± 5.64 pg/mL) and RRMS (IL-6: 15.21 ± 4.57 pg/mL; TNF-α: 23.98 ± 6.41 pg/mL). 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 the one-way ANOVA further showed significant differences among subtypes for IL-6 (p = 0.038) and TNF-α (p = 0.042); IgA and IgG did not show significant differences (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 disease duration, the higher the IgA levels (p = 0.041); no such correlation was observed for IgG (p = 0.089).

Consistent with the pattern of increasing disease severity, TNF-α and IL-6 concentrations were lowest in RRMS, intermediate in PPMS, and highest in SPMS (Figure 3).

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."

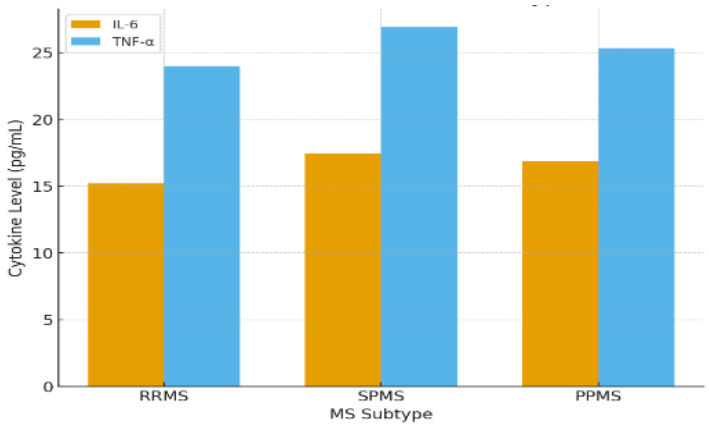

Figure 3. Showing comparison of IL-6 and TNF-α levels across RRMS, SPMS, and PPMS.

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Discussion

In this research, Iraqi MS patients exhibit increased serum levels of immunoglobulins (IgA, IgG) and elevated pro-inflammatory cytokines (IL-6, TNF-α), with significant positive correlations 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 pro-inflammatory 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 consistent with the global view that MS is a disease of both innate and adaptive immunity (18,19). Still, 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, disease duration, and MS subtype, further confirmed strong positive correlations between IL-6, TNF-α, immunoglobulins, and disability.

According to these analyses, TNF-α levels were independently associated with IgA levels and a progressive disease phenotype, whereas IL-6 levels were independently associated with IgG levels and disability (EDSS score). Importantly, in addition to disease duration, both cytokines were independent predictors of neurological disability (EDSS).

These regression models support the conclusion that the observed associations are not merely epiphenomena of broader disease activity or duration. Rather, they imply that these immune mediators have a more direct, possibly contributing role in the humoral response and disease development 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 Iraq's particular genetic and environmental context, including endemic mucosal pathogens and specific HLA alleles that influence cytokine production.

Additionally, the finding that both cytokines independently contribute to the EDSS score underscores their role as potential drivers of cumulative neurological damage, in addition to serving as indicators of inflammation. This strengthens the case for their study as therapeutic targets, especially for progressive forms of the illness, where few treatments are available.

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

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in both IgA and IgG levels alongside proinflammatory cytokines, might reflect a synergistic effect arising from environmental and genetic factors specific to 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 stronger association we found between TNF-α and IgA in this cohort, compared with previous studies, may indicate a greater influence of the mucosal and systemic immune compartments in this population, possibly 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, circulating levels of IL-6 and TNF-α were higher in patients with MS than in healthy controls (Table 2). This observation suggests increased systemic inflammation in MS, consistent with the known pathophysiology characterized by chronic immune-mediated demyelination and neurodegeneration (20).

- Elevated levels of IL-6 and TNF-α in MS cases have also been reported in previous studies across European and Asian populations. For instance, Strijbis, E. M. M., & Koch, M. W. observed elevated TNF-α levels in patients with remitting-remitting MS (RRMS) (15). In contrast, Park, E., & Ciofani, M. observed that serum IL-6 concentration mirrors clinical disease activity (21). Therefore, these findings underscore the role 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 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, an endemic characteristic in Iraq, may induce upregulation of systemic inflammatory mediators (14,22).

Stage of disease: Patients had not received immunosuppressive therapy shortly before the study; however, the confounding suppression of cytokine levels is minimized.

Taken together, these mechanisms may contribute to the observed increases in IL-6 and TNF-α, suggesting that these cytokines are candidate biomarkers of immune activation in MS.

In addition to the cytokine findings, as shown in Table 3, the study reported 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 has been reported to facilitate B-cell differentiation into plasma cells and to enhance antibody production (23). Accordingly, the positive relationships between IL-6/TNF-α and IgA/IgG reported in this study (Table 4; $p < 0.01$) support these associations and indicate that systemic inflammation directly drives humoral immune activation in MS.

Therefore, the positive association between IL-6 and IgG ($r = 0.418$, $p = 0.001$) is not merely an

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associative finding but rather an indicator of a direct pathophysiological driver in MS; IL-6 is the central factor driving B cells to differentiate into antibody-producing plasma cells (23). This suggests the presence of active IL-6-mediated humoral immunity. Increases in serum IgG, 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, as supported by recent experimental and early clinical trial work (24).

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

According to our stratified analysis, serum levels of TNF-α and IL-6 were significantly higher in patients with SPMS and PPMS than in those with RRMS ($p < 0.05$), as shown in Table 7. The idea that progressive multiple sclerosis is defined by a shift 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 explanations for why these cytokines are consistently elevated in progressive disease. Resident microglia and astrocytes in the central nervous system (CNS) are a continuous source of IL-6 and TNF-α, which drive 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 support their roles in 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 the chronic cytokine milieu may influence, solidifies over time. This aligns with more recent research showing that 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 previous studies in Eastern and Western populations, suggesting that MS pathobiology may be largely preserved across ethnic groups (15,21).

While immunoglobulins (IgG) in cerebrospinal fluid (CSF) and oligoclonal bands remain classic markers, slightly elevated serum IgG levels, though modest, have been described in individuals with MS, 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 associated with both environmental and immunological conditions (26).

Increased levels of both cytokines and immunoglobulins in this group suggest a synergistic model in which proinflammatory cytokines

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crosstalk to promote B-cell activity, leading to elevated immunoglobulins and, subsequently, disease exacerbation. However, the most notable finding in our study, as shown in Table 4, was the strong correlation between TNF-α and IgA ($r = 0.496$, $p < 0.001$), which exceeds what is commonly observed in Western cohorts. We speculate that local environmental conditions could modulate this immunological signature. The endemicity of certain mucosal pathogens in Iraq may lead to chronic mucosal immune activation (24), in which TNF-α-induced inflammation and barrier defense could, in turn, continuously drive IgA production. This is particularly relevant given that the gut-brain axis is increasingly 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 individuals 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 warrants further research through focused serological and microbiome studies.

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

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, the exceptionally strong correlation between TNF-α and IgA, along with the finding that patients with progressive disease phenotypes have the highest levels of these markers, suggests a possible population-specific immunopathological axis. We propose that endemic environmental factors, such as prolonged exposure to mucosal pathogens, may prime systemic and possibly CNS-compartmentalized humoral immunity via the gut-brain axis (17,27).

Although these inflammatory pathways are known to occur in multiple sclerosis worldwide (15,21), the unique pattern observed here highlights the crucial roles of regional and genetic factors in disease mechanisms. Thus, in addition to confirming the roles 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 region-specific biomarker studies.

Limitations and Future Directions

Although our study provides important insights into the immune profile of Iraqi patients with multiple sclerosis, it has several limitations. The lack of correlation between biomarker levels and recent clinical disease activity (e.g., relapse rate or time since last relapse) is a significant

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limitation. As a result, we cannot determine 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. Second, although the sample size for statistical analysis was acceptable, larger multicenter populations may help generalize the current results across different Iraqi regions and ethnic groups. Third, only blood-based biomarkers were evaluated; adding 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 adequately adjusted for, which may have affected immunoglobulin levels, especially IgA.

Lastly, genetic and environmental risk factor analyses should be combined with serial immunological profiling of serum and CSF in future studies. This approach would help determine the predictive value of TNF-α, IgA, IgG, and IL-6 for aggressive disease courses. Demonstrating a quantifiable improvement in disease course would eventually allow intervention trials targeting these cytokines to 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 ethics committee of the Middle Technical University, Iraq (2603, 31/12/2024).

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