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Original Article

Serum Interleukin-40 and Soluble CD40 Ligand as Complementary Biomarkers for Disease Activity in Multiple Sclerosis Patients

Multipl Skleroz Hastalarında Hastalık Aktivitesinin Tamamlayıcı Biyobelirteçleri Olarak Serum

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ABSTRACT

Objectives: Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system for which reliable biomarkers of disease activity remain an unmet need. Interleukin-40 (IL-40) and soluble CD40 ligand (sCD40L) have been proposed to play roles in the pathogenesis of autoimmune diseases. This study aimed to evaluate serum levels of IL-40 and sCD40L as biomarkers of disease activity in MS patients, and to compare their diagnostic and monitoring performance between MS patients and healthy controls.

Methods: One hundred twenty MS patients were recruited from the Department of MS at the Baghdad Teaching Hospital and divided into two groups based on disease status: active (n=60) and inactive (n=60). Additionally, 57 matched healthy individuals were included as controls. A sandwich enzyme-linked immunosorbent assay was used to measure the serum levels of IL-40 and sCD40L in blood samples from each participant.

Results: Both active and inactive patient cohorts showed significantly higher serum levels of IL-40 (44.25±8.57 ng/mL and 38.98±11.31 ng/mL, respectively) compared with their control group (20.82±14.27 ng/mL) (p=0.005). Likewise, sCD40L concentrations were elevated in both active (2155.59±587.02 pg/mL) and inactive (1885.23±851.32 pg/mL) patients compared with controls (849.79±341.87 pg/mL; p=0.0006). IL-40 correlated positively with sCD40L (r=0.399, p=0.005). The receiver operating characteristic analysis showed high diagnostic performance for IL-40 (area under the curve =0.873; sensitivity 87.5%; specificity 76.7%) and sCD40L (area under the curve =0.901; sensitivity 92.5%; specificity 81.7%).

Conclusions: Both IL-40 and sCD40L are significantly elevated in MS and exhibit promising diagnostic validity. These biomarkers may serve as complementary tools for monitoring MS disease activity and progression, offering potential value in clinical practice and therapeutic decision-making.

Keywords: Multiple sclerosis, interleukin-40, soluble CD40 ligand, biomarker, disease activity

ÖZ

Amaç: Multipl skleroz (MS), merkezi sinir sisteminin karmaşık bir otoimmün hastalığıdır ve hastalık aktivitesinin güvenilir biyobelirteçleri hala karşılanmamış bir ihtiyaçtır. Interlökin-40 (IL-40) ve çözünür CD40 ligandının (sCD40L) otoimmün hastalıkların patogenezinde rol oynadığı öne sürülmüştür. Bu çalışma, MS hastalarında hastalık aktivitesinin biyobelirteçleri olarak IL-40 ve sCD40L serum düzeylerini değerlendirmek ve MS hastaları ile sağlıklı kontrol grubu arasında bu belirteçlerin tanı ve izleme performansını karşılaştırmak amacıyla yapılmıştır.

Yöntemler: Bağdat Eğitim Hastanesi MS Bölümünden 120 MS hastası seçildi ve hastalık durumuna göre aktif (n=60) ve inaktif (n=60) olmak üzere iki gruba ayrıldı. Ayrıca, 57 eşleştirilmiş sağlıklı birey kontrol grubu olarak dahil edildi. Her katılımcının kan örneklerinde IL-40 ve sCD40L serum düzeylerini ölçmek için sandviç enzim bağlı immünosorbent testi kullanıldı.

Bulgular: Hem aktif hem de inaktif hasta kohortları, kontrol grubuna (20,82±14,27 ng/mL) kıyasla (p=0,005) anlamlı olarak daha yüksek IL-40 serum düzeyleri (sırasıyla 44,25±8,57 ng/mL ve 38,98±11,31 ng/mL) gösterdi. Benzer şekilde, sCD40L konsantrasyonları, aktif (2155,59±587,02 pg/mL) ve inaktif (1885,23±851,32 pg/mL) hastalarda kontrol grubuna (849,79±341,87 pg/mL; p=0,0006) kıyasla yükselmiştir. IL-40, sCD40L ile pozitif korelasyon gösterdi (r=0,399, p=0,005). Alıcı işletim karakteristiği analizi, IL-40 (eğri altındaki alan =0,873; duyarlılık %87,5; özgüllük %76,7) ve sCD40L (eğri altındaki alan =0,901; duyarlılık %92,5; özgüllük %81,7) için yüksek tanısal performans gösterdi.

Sonuçlar: Hem IL-40 hem de sCD40L, MS'te önemli ölçüde yükselmiştir ve umut verici tanısal geçerlilik sergilemektedir. Bu biyobelirteçler, MS hastalığının etkinliğini ve ilerlemesini izlemek için tamamlayıcı araçlar olarak hizmet edebilir ve klinik uygulamada ve tedavi kararlarında potansiyel değer sunabilir.

Anahtar kelimeler: Multipl skleroz, interlökin-40, Çözünür CD40 ligand, biyobelirteçler, hastalık etkinliği

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INTRODUCTION

Multiple sclerosis (MS) is a chronic disorder of the central nervous system (CNS) that involves immune-mediated attack on CNS tissues, leading to inflammation, demyelination, and nerve damage[1,2]. The most common type is relapsing-remitting multiple sclerosis (RRMS), which affects both adults and children[3]. This form is characterized by periods of worsening neurological symptoms followed by partial or full recovery[1]. Since the disease can progress in variable and unpredictable ways, researchers are developing more accurate diagnostic and predictive tests for MS.

Research advances in the field of immunology have led to a significant reshaping of the understanding of the pathophysiology of MS[4], which is thought to encompass genetic susceptibility, environmental triggers, and dysregulation of immune responses leading to CNS tissue damage[5]. A central focus of research in this area has been the identification of biomarkers that aid in the diagnosis[6] and prognosis[7] of the disease, and that guide possible therapeutic approaches[8]. Neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) have been linked to axonal injury[9] and astrocyte injury[10]. However, their ability to distinguish between the activity phases of MS is limited[11]. Cytokines and chemokines have been identified as potentially useful biomarkers for MS due to their roles in immune cell activation, trafficking, and communication[12]. Early studies have linked chemokine ligand 13, interleukin-6 [(IL)-6], and IL-17 to inflammation and disease progression[13], but their specificity in MS remains uncertain. Despite research into biomarkers for MS, including serum glutamate[14] and GFAP[15] as potential predictors of disease activity, there is a lack of consensus on any single biomarker with sufficient sensitivity and specificity to be used routinely in clinical practice to diagnose and monitor disease progression in patients with MS[16].

B cell biology is a pivotal component of the immunopathology of MS[17], and B cell-depleting therapies are therapeutically effective[18]. Advances in B cell research have led to studies on associated molecules, including IL-40, which is known to be involved in the maturation of B cells and in immune regulation[19, 20], and soluble CD40 ligand (sCD40L), which is a co-stimulatory molecule known to mediate crosstalk between T and B cells[21]. Research in these areas may increase understanding of inflammatory activity and immune dysregulation in MS. Evaluating IL-40 and sCD40L within the clinical context could therefore further elucidate biomarkers in MS and improve disease monitoring, risk stratification, and risk mitigation in MS patients.

IL-40 has been identified as a potential biomarker in autoimmune diseases, including rheumatoid arthritis[22] and systemic lupus erythematosus[23], suggesting a pro-inflammatory role for IL-40, given its association with increased disease activity. Elevated levels of sCD40L have been reported in inflammatory and autoimmune diseases[24] and correlate with immune activation and disease severity. In MS patients, the CD40-CD40L pathway has been reported to contribute to T-cell priming and the amplification of CNS-directed autoimmunity[25], suggesting that sCD40L is a potentially reliable marker of immune activation during MS relapses[26].

MS has long been considered primarily driven by T cells. However, recent advances in immunology and pathology have uncovered novel insights into the pathogenesis of MS, shedding light on the role of B cells. Given that IL-40 is mainly secreted by activated B cells and sCD40L is a co-stimulatory molecule that enhances B cell activation and antibody production, studying these markers may provide complementary insights into B cell-related immune dysregulation in MS and improve the accuracy of assessing disease activity.

To the best of our knowledge, this is the first study to simultaneously investigate IL-40 and sCD40L as complementary biomarkers for MS activity assessment. This study aims to examine the association between IL-40, sCD40L, and disease activity in MS patients. Evaluating these potential biomarkers could improve understanding of the immunopathology of MS and provide additional means to assess disease activity and progression, particularly in patients with atypical disease progression.

MATERIALS and METHODS

This cross-sectional study involved 177 participants (both males and females), aged 18-63 years, recruited from Baghdad Teaching Hospital between October 2024 and March 2025. In this cohort, 120 patients were diagnosed with MS according to the McDonald criteria 2017[27].

Patients were divided into two subgroups based on their disease activity according to No Evidence of Disease Activity-3 (NEDA-3) criteria (28): active (n=60) and inactive (n=60). The remaining 57 subjects constituted the control group of apparently healthy individuals age- and sex-matched to the study participants. All inactive MS patients were in remission period without any clinical relapses or new magnetic resonance imaging (MRI) lesions for at least 12 months.

Active MS patients (n=60) were defined according to NEDA-3 criteria as those showing at least one of the following: (a) clinical relapse confirmed by a neurologist, (b) new or enlarging T2 lesions on MRI, or (c) disability progression. Among active patients were newly diagnosed patients presenting with recent attacks and previously diagnosed patients who experienced relapses and/or MRI activity. Exclusion criteria included other autoimmune or inflammatory diseases, pregnancy or lactation, and lack of voluntary patient consent to participate in the study.

The demographic data for all participants and the clinical data for patients (last relapse, Expanded Disability Status Scale (EDSS), disease type, disease duration, and family history) were recorded. Written informed consent was obtained from all participants. The study was approved by the Scientific Committee of Ethics at the College of Medicine, University of Baghdad (approval number: 0254, date: 21.09.2025).

Body mass index (BMI) was recorded for all participants to evaluate any potential association between body composition and serum cytokine concentrations, given that adipose tissue is known to produce inflammatory mediators that may influence immune responses in autoimmune conditions.

A 5-mL venous blood sample was obtained from each participant. Serum was separated and stored at -20 °C until the time of analysis. Human sandwich IL-40 and sCD40L enzyme-linked immunosorbent assay (ELISA) kits were used according to the manufacturer's instructions. ELISA plates pre-coated with antibodies specific to either IL-40 or sCD40L were employed. Samples and standards were added to the predetermined wells and incubated for 80 minutes at 37 °C. Any unbound substances were removed by washing three times with an automatic plate washer. Biotinylated antibodies specific to either IL-40 or sCD40L were added and incubated for 50 minutes at 37 °C. After incubation and washing, streptavidin-HRP was added, and the mixture was incubated for 50 minutes at 37 °C. Plates were washed an additional five times before adding the tetramethylbenzidine substrate solution. The reaction was stopped by adding the stop solution, and the absorbance was measured at 450 nm using a microplate reader. All samples and standards were run in duplicate to ensure assay reliability. The ELISA kits (catalogue numbers ELK0969 for IL-40 and ELK9196 for sCD40L) were obtained from ELK Biotechnology (China).

Statistical Analysis

The Statistical Package for the Social Sciences (version 26, IBM, Armonk, NY, USA) was used for the statistical analysis. Microsoft Office Excel 2010 (Microsoft Corporation, Redmond, WA, USA) was used to produce all figures except for the receiver operating characteristic (ROC) curve.

Normality testing was performed before applying the parametric tests [analysis of variance (ANOVA) and t-test] using the Kolmogorov-Smirnov test. Normally distributed data are expressed as mean ± standard deviation (SD). Independent-samples Student's t-test, ANOVA, and least significant difference (S) tests were performed to compare quantitative variables among study groups (age, years), BMI, disease duration, EDSS, serum IL-40 (ng/mL), and sCD40L (pg/mL). The Pearson chi-square test (χ^2) was applied only to categorical variables such as sex, smoking status, MS type, and family history.

Test validity was estimated using ROC curve analysis, the cut-off value, the area under the curve (AUC), sensitivity (%), specificity (%), positive predictive value (PPV), negative predictive value (NPV), and accuracy. The statistical significance threshold (p-value) was defined as follows: $p>0.05$ for a non-S, $p<0.05$ for a S, and $p<0.01$ for a highly significant difference (HS).

RESULTS

Table 1 summarizes the demographic data. One hundred twenty patients with MS (active =60; inactive =60) were aged 18–63 years, and the mean age of active MS patients was slightly higher than that of inactive MS patients and controls. The remaining participants (n=57) were apparently healthy controls, aged 19-58 years (mean age 32.77±10.43 years). Across all cohorts studied, female participants outnumbered male participants [female MS group: n=78 (65%); female control group: n=34 (59.6%)]. Statistical analysis revealed no Ss in age (p=0.673) or sex (p=0.516) among the studied groups (i.e., the healthy controls were age- and sex-matched).

Age was categorized into four groups: 18-30, 31-40, 41-50, and 51-63 years. Nearly half of the patients (n=50, 41.6%) and more than one-third of the controls (n=21, 36.8%) were aged 18-30 years. Similar to age, the BMI was categorized as normal weight, overweight, or obese using the standard BMI classification (normal: 18.50-24.9, overweight: 25.0-29.9, obese: 6gt; ≥30). The frequencies of overweight among active MS patients (n=27, 45%) and healthy controls (n=25, 43.9%) were elevated. However, nearly half of the inactive patients (n=27, 45%) fall into the normal-weight category. No statistically S was observed (p=0.495). The mean BMI of active MS patients (27.05±4.64 kg/m^2), inactive MS patients (26.20±4.50 kg/m^2), and the control group (26.57±4.16 kg/m^2) did not differ significantly (p=0.575).

Table 1. Distribution of demographic and other data among study groups.

Parameters		Activity			p-value
		Control (C) n=57	Inactive (I) n=60	Active (A) n=60	
Smoking	Smokers	12 (21.1%)	13 (21.7%)	14 (23.3%)	p=0.911
	Non-smokers	45 (78.9%)	47 (78.3%)	46 (76.7%)	
Sex	Male	23 (40.4%)	20 (33.3%)	22 (36.7%)	p=0.516
	Female	34 (59.6%)	40 (66.7%)	38 (63.3%)	
Age groups /year	18-30	21 (36.8%)	27 (45%)	23 (38.3%)	p=0.673
	31-40	18 (31.6%)	19 (31.7%)	18 (30%)	
	41-50	14 (24.6%)	10 (16.7%)	17 (28.3%)	
	51-63	4 (7%)	4 (6.7%)	2 (3.3%)	
BMI Groups	Normal weight	21 (36.8%)	27 (45%)	19 (31.7%)	p=0.495
	Overweight	25 (43.9%)	23 (38.3%)	27 (45%)	
	Obese	11 (19.3%)	10 (16.7%)	14 (23.3%)	
Age / year	Mean	32.77	33.83	34.71	C-I- p=0.563
	Standard deviation	10.427	10.484	9.323	C-A - p=0.295
	Standard error	1.346	1.353	1.204	I-A - p=0.639
	ANOVA test (p-value): p=0.578				
BMI /kg/m^2	Mean	26.5667	26.2001	27.0517	C-I - p=0.651
	SD	4.15939	4.50326	4.63829	C-A - p=0.551
	Standard error	0.53698	0.58137	0.5988	I-A - p=0.296
	ANOVA test (p-value): p=0.575				

p>0.05: Non-significant difference, BMI:Body mass index, SD: Standard deviation, ANOVA: Analysis of variance.

Non-smokers predominated among active MS patients (n=46, 76.7%), inactive MS patients (n=47, 78.3%), and controls (n=45, 78.9%); no S was observed (p=0.911).

Table 2 presents the clinical data for MS patients. In the active group, 39 patients (65%) were newly diagnosed following recent relapses, while 21 patients (35%) had an MS diagnosis established at least 1 year ago and presented with either a relapse or MRI activity. None of the inactive patients were newly diagnosed; all had been diagnosed with MS for at least 1 year, and a HS was observed (p=0.004). The majority of patients (49 active and 52 inactive) were diagnosed with RRMS, followed by primary progressive multiple sclerosis (PPMS) and secondary progressive multiple sclerosis (SPMS); there was no statistically S between the active and inactive groups (p=0.253). A limited number of patients (5 active and 7 inactive) had a family history of MS, but this difference was not statistically significant (p=0.543).

There was a statistically S in disease duration (p=0.008), with inactive patients having longer disease duration than active patients.

Lastly, the mean EDSS scores of the two patient cohorts are similar, with no statistically observed (p=0.291).

Table 3 summarizes the mean serum biomarker levels across all study groups. Both active and inactive MS patients showed higher serum IL-40 concentrations than healthy controls, with a statistically S (p=0.005). The active group had slightly but statistically significantly higher serum concentrations of IL-40 than the inactive group (p=0.014).

Likewise, the mean serum concentrations of sCD40L of active MS patients were higher than inactive, and control groups (p=0.0006).

There was no S (p=0.236) in the serum levels of the study markers among the three types of MS (Table 4). Serum levels of IL-40 in patients diagnosed with SPMS are lower than those in patients diagnosed with RRMS and PPMS. Similarly, serum sCD40L levels in patients with SPMS are lower than those in patients with RRMS and PPMS (p=0.434).

In this study, the vast majority of patients were taking medications for MS. No Ss was observed in

Table 2. Clinical characteristics of MS patients according to disease activity.				
Parameters		**Activity**		**p-value**
		Inactive N=60	**Active N=60**	
Newly diagnosed	Yes	0 (0%)	39 (65%)	**p=0.004**
	No	60 (100%)	21 (35%)	
Family history	Yes	7 (11.7%)	5 (8.3%)	p=0.543
	No	53 (88.3%)	55 (91.7%)	
Type of MS	SPMS	3 (5%)	1 (1.7%)	p=0.253
	RRMS	52 (86.7%)	49 (81.7%)	
	PPMS	5 (8.3%)	10 (16.7%)	
Duration/ Year	Mean	7.6	3.57	**p=0.008**
	SD	4.85	4.82	
	Standard error	0.63	0.62	
EDSS	Mean	1.86	1.52	p=0.291
	Standard deviation	2.07	1.39	
	Standard error	0.27	0.18	

p>0.05: Non-significant difference, p<0.01: Highly significant difference, MS: Multiple sclerosis, EDSS: Expanded disability status scale, SD: Standard deviation, SPMS: Secondary progressive multiple sclerosis, RRMS: Relapsing-remitting multiple sclerosis, PPMS: Primary progressive multiple sclerosis, ANOVA: Analysis of variance.

Table 3.Mean distributions of serum IL-40 and sCD40L among study groups according to the activity of MS.						
Activity of MS		**Mean**	**Standard deviation**	**Standard error**	**p-value**	
IL-40 (ng/mL)	Control (C)	20.82	14.27	1.84	C-I	**p=0.003**
	Inactive (I)	38.98	11.31	1.46	C-A	**p=0.001**
	Active (A)	44.25	8.57	1.11	I-A	**p=0.014**
	ANOVA test (p-value): **p=0.005**					
sCD40L (pg/mL)	Control (C)	849.79	341.88	44.14	C-I	**p=0.005**
	Inactive (I)	1885.23	851.32	109.91	C-A	**p=0.0002**
	Active (A)	2155.59	587.02	75.78	I-A	**p=0.021**
	ANOVA test (p-value): **p=0.0006**					

p<0.05: Significant difference, p<0.01: Highly significant difference, MS: Multiple sclerosis, IL-40: Interleukin-40, sCD40L: Soluble cluster of differentiation 40 ligand, ANOVA: Analysis of variance.

serum levels of IL-40 and sCD40L in most comparisons between MS patients receiving treatment and those not receiving treatment; mean ± standard deviation values were similar. However, there are exceptions for those taking Natalizumab (p=0.044), Avonex (p=0.047), and Fingolimod (p=0.009). Correlation analyses of patient parameters also revealed a positive, statistically significant association between serum IL-40 and sCD40L levels in patients with MS (r=0.399, p=0.005). In contrast, all other correlations were weak, either positive or negative, and not statistically significant (p>0.05) (Table 5).

Sensitivity and Specificity Analysis

ROC curves show that IL-40 demonstrates good diagnostic validity at a cut-off value of 28.5 ng/mL, with an AUC of 0.873 [95% confidence interval (CI), 0.81-0.92] and high sensitivity (87.5%), specificity (76.7%), PPV (88.2%), NPV (75.4%), and accuracy (83.89%) (p=0.0005) (Figure 1).

Additionally, sCD40L demonstrated excellent validity for the diagnosis and follow-up of MS patients of, with an AUC of 0.901 (95% CI, 0.85-0.94), high sensitivity (92.5%), good specificity (81.7%), PPV (91%), NPV (84.5%), and accuracy (88.89%) (p<0.0002) (Figure 2).

Table 4. Mean distributions of serum IL-40 and sCD40L in MS patients according to the type of MS.						
Assays	**Type of MS**	**N**	**Mean**	**Standard deviation**	**Standard error**	**p-value**
IL-40 (ng/mL)	SPMS	4	32.1	6.94	3.47	p=0.236
	RRMS	101	41.86	10.62	1.06	
	PPMS	15	42.28	8.41	2.17	
	Total	120				
sCD40L (pg/mL)	SPMS	4	1753.13	935.58	467.79	p=0.434
	RRMS	101	2001.23	746.08	74.24	
	PPMS	15	2220.67	658.49	170.02	
	Total	120				

p>0.05: Non-significant difference, MS: Multiple sclerosis, IL-40: Interleukin-40, sCD40L: Soluble cluster of differentiation 40 ligand, SPMS: Secondary progressive multiple sclerosis, RRMS:Relapsing-remitting multiple sclerosis, PPMS: Primary progressive multiple sclerosis.

Table 5. Correlation study between the parameters of MS patients.			
Pearson Correlation (MS patients)		**IL-40 (ng/mL)**	**sCD40L (pg/mL)**
sCD40L (pg/mL)	r	0.399	
	p-value	0.005	
	Sign.	HS	
Age/year	r	0.031	0.146
	p-value	0.737	0.111
	Sign.	NS	NS
BMI (Kg/m^2)	r	-0.027	0.102
	p-value	0.772	0.266
	Sign.	NS	NS
Duration / Year	r	0.148	0.025
	p-value	0.107	0.785
	Sign.	NS	NS
EDSS	r	-0.026	0.051
	p-value	0.776	0.578
	Sign.	NS	NS

p>0.05: Non-significant difference (NS), p<0.01: Highly significant difference (HS), MS: Multiple sclerosis, IL-40: Interleukin 40, sCD40L: Soluble cluster of differentiation 40 ligand, BMI: Body mass index, EDSS: Expanded disability status scale, Sign: Significance, r:correlation coefficient.

DISCUSSION

This study has shown that both IL-40 and sCD40L are significantly elevated in patients with MS compared to healthy controls. Patients with active disease had particularly high concentrations of both IL-40 and sCD40L. These two biomarkers were positively correlated, with both showing robust diagnostic validity in ROC analyses, and with sensitivities and specificities comparable to those of established candidate biomarkers. This suggests that IL-40 and sCD40L could be useful biomarkers for the clinical evaluation of MS activity and disease progression.

The positive correlation observed between IL-40 and sCD40L in this study may reflect their complementary roles in immune regulation. IL-40 is known to be a B cell–associated cytokine involved in the production of immunoglobulins as well as the modulation of humoral responses. sCD40L is biologically active and can engage CD40 expressed on B cells. Their concurrent elevation in active MS suggests a possible contributing role in immune activation and subsequent disease activity[19,25].

Elevated serum concentrations of IL-40 may reflect enhanced B cell activation, whereas higher levels of sCD40L may indicate an ongoing T cell-mediated immune response. Therefore, their combined profiling could serve as a helpful tool for assessing disease progression risk and monitoring therapeutic response, specifically sCD40L, which has been reported to decrease after treatment[26,29,30]. However, further longitudinal studies are required to validate their predictive utility in clinical practice.

The results of this study are consistent with previous research implicating the CD40-CD40L axis in MS pathogenesis[25]. Elevated levels of sCD40L have been reported in SPMS and have been linked to immune activation through B cell and T cell interactions[31]. Studies in animal models of experimental autoimmune encephalomyelitis have shown that blocking the CD40-CD40L pathway leads to attenuation of CNS inflammation and demyelination[32], indicating the mechanistic relevance of this pathway to immune activation. The study adds to these observations by demonstrating that sCD40L levels are elevated in progressive MS and in actively relapsing patients, suggesting that they are a dynamic marker of immune activity.

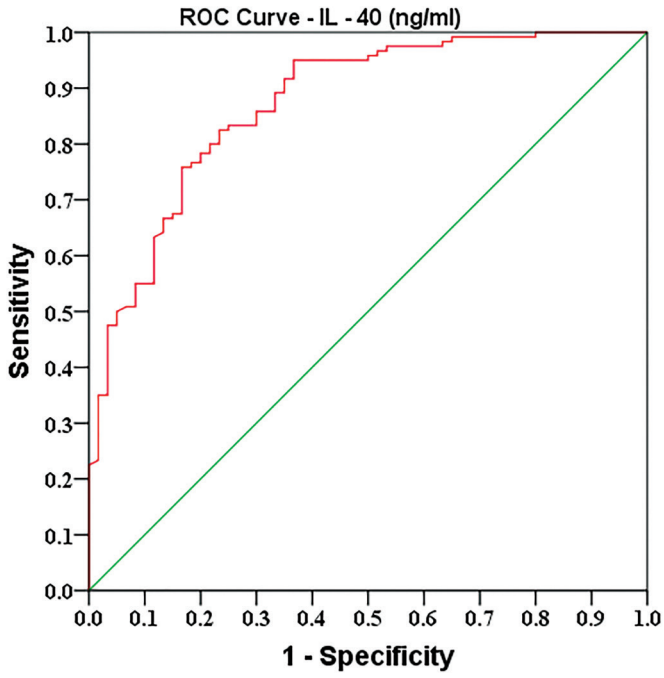

Figure 1. Validity tests of IL-40 by using ROC test in sera of MS patients and controls.

MS: Multiple, ROC: receiver operating characteristic, IL-40: Interleukin-40

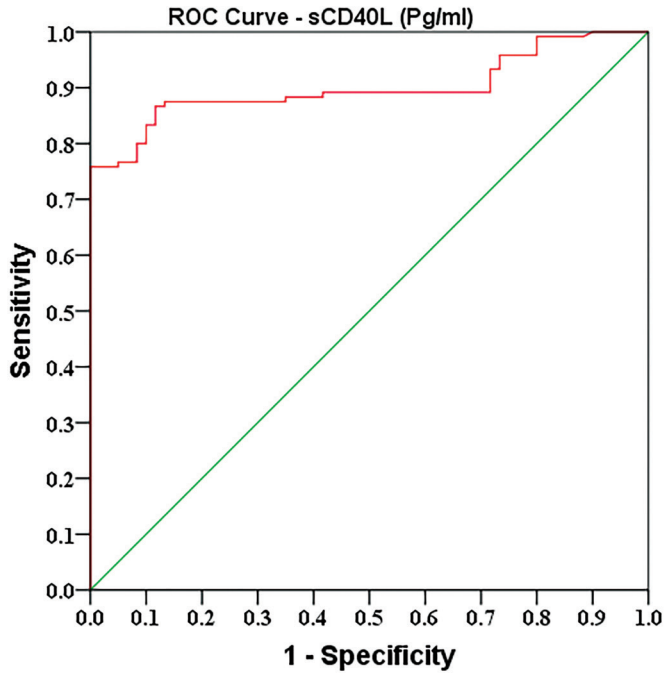

Figure 2. Validity tests of sCD40L by using ROC test in sera of MS patients and controls.

ROC: receiver operating characteristic, sCD40L: Soluble cluster of differentiation 40 ligand, MS: Multiple

IL-40 is a biomarker associated with immunoglobulin production and mucosal immunity through B cell homeostasis[33]. Subsequent research has reported elevated IL-40 levels in rheumatoid arthritis[34] and systemic lupus erythematosus[23], with IL-40 levels correlating with disease activity. The results of this study, showing higher IL-40 concentrations in active versus inactive MS patients, support the assertion that IL-40 may be an activity-related biomarker that complements markers of axonal damage, such as NfL.

The high sensitivity and specificity observed for IL-40 and sCD40L in distinguishing MS patients from healthy controls are clinically relevant findings. Currently, no single biomarker is recommended for the diagnosis of MS; diagnosis remains based on clinical and MRI-based assessments. Biomarkers with strong diagnostic validity could offer adjunctive value, especially in atypical presentations of MS or in settings where advanced neuroimaging is not widely available. Beyond diagnosis, the identified differential expression of IL-40 and sCD40L could signify impending relapses or subclinical inflammatory activity, thereby guiding decisions about therapeutic interventions and monitoring frequency for such patients.

The results from the subgroup analysis presented in this study suggest that disease-modifying therapies, such as Natalizumab, Avonex, and Fingolimod, can all influence biomarker levels. Notably, patients receiving Fingolimod exhibited reduced sCD40L levels, reinforcing its role as a modulator of lymphocyte trafficking[26]. This finding suggests that IL-40 and sCD40L could be useful in evaluating treatment response, allowing the stratification of patients who are likely to benefit from specific therapeutic approaches.

The novelty of this study lies in its combined evaluation of IL-40 and sCD40L; although both biomarkers have been investigated separately in the context of autoimmune diseases, limited research has examined their interplay in MS. The positive correlation between these two biomarkers observed in this study suggests that they may have a shared or complementary role in disease activity. This approach, using two biomarkers, may therefore be more beneficial than single biomarkers for understanding MS heterogeneity and disease progression.

The results of the present study can lay the foundation for future longitudinal research tracking IL-

40 and sCD40L concentrations in MS patients across different disease phases. Accordingly, researchers might determine whether changes in IL-40 and sCD40L levels precede relapses or the onset of new MRI-detected inflammation. This will reflect the interplay of B and T cells that perpetuates CNS inflammation[35,36]. Future studies combining cytokine-level measurements with brain imaging and treatment-response data could help clarify how IL-40 and sCD40L affect MS, thereby facilitating more personalized care.

Study Limitations

This study has several limitations. First, the study used a cross-sectional design and did not undertake longitudinal monitoring, which may have limited the study's ability to determine whether IL-40 and sCD40L levels fluctuate predictably prior to clinical relapses. Additionally, the potentially confounding effects of disease-modifying therapies on biomarker levels cannot be excluded in this study. Despite these limitations, our study provides important preliminary evidence supporting IL-40 and sCD40L as complementary biomarkers in MS, warranting validation in larger, longitudinal, and multi-center investigations.

CONCLUSION

The current study reports significantly elevated levels of IL-40 and sCD40L in MS patients compared with controls, indicating their potential utility as diagnostic biomarkers, and higher levels in patients with active MS than in those with inactive MS, indicating their potential utility in disease monitoring. Positive ROC results indicate promising clinical utility. However, multi-center and longitudinal studies are needed to validate these findings and to explore the predictive value of IL-40 and sCD40L for monitoring disease relapse and progression before integrating these biomarkers into multi-biomarker panels to deliver precision medicine to MS patients.

Ethics

Ethics Committee Approval: The study was approved by the Scientific Committee of Ethics at the College of Medicine, University of Baghdad (approval number: 0254, date: 21.09.2025).

Informed Consent: Written informed consent was obtained from all participants.

Footnotes

Author Contributions

Surgical and Medical Practices: G.A.G., Concept: M.Z.N., I.K.S., G.A.G., Design: M.Z.N., I.K.S., G.A.G., Data Collection and/or Processing: M.Z.N., G.A.G., Analysis or Interpretation: M.Z.N., I.K.S., G.A.G., Literature Search: M.Z.N., I.K.S., G.A.G., Writing: M.Z.N., I.K.S.,

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