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Research Article

Serum Neurofilament Light Chain as an Independent Biomarker of Axonal Damage of Psychiatric Burden in Multiple Sclerosis

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Abstract

Background: Multiple sclerosis (MS) is a chronic neuroinflammatory disorder associated with neurological disability and psychiatric comorbidity. Serum neurofilament light chain (sNfL) is a biomarker of axonal injury, yet its relationship with psychiatric symptoms in MS is unclear. ***Objective***: To assess depression and anxiety prevalence in Iraqi MS patients and the associations with sNfL. ***Methods***: A cross-sectional case–control study was conducted at the MS Consultation Clinic, Iraqi Medical City, Baghdad (October 2024–January 2025). Depression and anxiety were evaluated with the Hospital Anxiety and Depression Scale (HADS), disability with the Expanded Disability Status Scale (EDSS), and sNfL with Enzyme-Linked Immunosorbent Assay (ELISA). ***Results***: A total of 150 MS patients and 50 healthy subjects (controls) were enrolled. Depression and anxiety were evaluated with the Hospital Anxiety and Depression Scale (HADS), disability with the Expanded Disability Status Scale (EDSS), and serum NfL with ELISA. MS patients had significantly higher depression (31.3%) and anxiety (14.7%) than controls (4.0% and 10.0%; $p<0.001$). Depression correlated with lower socioeconomic and educational status, while female sex and marital status predicted higher anxiety. Median sNfL levels were elevated in MS (169.6 *vs*. 98.9 pg/mL; $p<0.001$) but showed only a weak negative correlation with depression ($r= –0.174$, $p=0.034$). ***Conclusions*:** Depression and anxiety are prevalent in Iraqi MS patients and strongly linked to sociodemographic and disability measures. While NfL, as a marker of axonal injury, had limited value for detecting psychiatric comorbidity, emphasizing the need for integrated neurological and mental health care.

Keywords: Anxiety; Biomarkers; Depression; Expanded disability status scale; Multiple sclerosis; Neurofilament light chain.

مستوى السلسلة الخفيفة للخيوط العصبية في المصل كمؤشر حيوي مستقل لتلف المحاور العصبية الناتجة عن العبء النفسي في مرض التصلب المتعدد

الخلاصة

الخلفية: التصلب المتعدد (MS) هو اضطراب التهابي عصبي مزمن يرتبط بالإعاقة العصبية والاعتلال النفسي المصاحب. يعد الاكتئاب والقلق أمرًا متكررًا ولكن غالبًا ما يتم تشخيصه بشكل ناقص. تعتبر السلسلة الخفيفة من الخيط العصبي في الدم (sNfL) علامة حيوية للإصابة بالمحور العصبي، إلا أن علاقتها بالأعراض النفسية في مرض التصلب العصب. المتعدد غير واضحة. **الهدف**: تقييم مدى انتشار الاكتئاب والقلق لدى مرضى التصلب المتعدد العراقيين واستكشفت الارتباطات مع sNfL. **الطرائق**: أُجريت دراسة الحالات والشواهد المقطعية في العيادة الاستشارية لمرض التصلب العصبي المتعدد، مدينة الطب العراقية، بغداد (أكتوبر 2024 - يناير 2025). **النتائج**: تم تسجيل ما مجموعه 150 مريضا بالتصلب المتعدد و50 من الأصحاء المتطابقين. تم تقييم الاكتئاب والقلق باستخدام مقياس القلق والاكتئاب في المستشفى(HADS) ، والإعاقة باستخدام مقياس حالة الإعاقة الموسع(EDSS) ، ومصل NfL باستخدام ELISA. كان لدى مرضى التصلب المتعدد اكتئاب أعلى بكثير (31.3%) وقلق (14.7%) مقارنة بالضوابط (4.0% و10.0%؛ $p<0.001$). وارتبط الاكتئاب بانخفاض الوضع الاجتماعي والاقتصادي والتعليمي، في حين تنبأ جنس الإناث والحالة الاجتماعية بارتفاع القلق. كانت مستويات sNfLالمتوسطة مرتفعة في مرض التصلب العصبي المتعدد (169.6 مقابل 98.9 بيكوغرام / مل؛ $p <0.001$) ولكنها أظهرت فقط وجود علاقة سلبية ضعيفة مع الاكتئاب ($r=-0.174$، $p=0.034$). **الاستنتاجات**: الاكتئاب والقلق منتشران لدى مرضى التصلب المتعدد العراقيين ويرتبطان بقوة بالمقاييس الاجتماعية والديموغرافية والإعاقة. في حين أكد sNfL دوره كعلامة على إصابة محور عصبي، كان له قيمة محدودة للكشف عن الاعتلال المشترك للأمراض النفسية، مع التركيز على الحاجة إلى رعاية صحية عصبية وعقلية متكاملة.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system characterized by inflammation, demyelination, and axonal injury [1]. In Iraq, a recent retrospective review of 4,355 cases reported a prevalence of 11.73 per 100,000, with a female-to-male ratio of approximately 2.18:1; incidence has also risen over recent years [2]. Vitamin D insufficiency has been associated with higher

susceptibility to MS and appears as a modifiable risk factor. In this context, sunlight exposure is the primary source of vitamin D, which is also received through diet and dietary supplements. Notably, it is one of the most common nutritional deficiencies worldwide [3-5]. MS imposes substantial neurological disability, but equally important are its "invisible" symptoms, including psychological comorbidities, which can significantly impair quality of life and disease outcomes [6,7]. A number of reasons, such as disease course, side effects of treatment, family history, and psychosocial difficulties, cause psychiatric disorders of MS. Collectively, these findings highlight the heavy psychosocial burden of MS and underscore the importance of accurate identification and care of mental illness in conjunction with neurological therapy [8]. Identifying biomarkers indicative of pathological processes associated with mood and cognitive alterations could help facilitate early and more customized therapeutic interventions [9]. Neurofilament light chain (NfL) has emerged as a promising biomarker for axonal injury [10]. It is a neuronal cytoskeletal protein released into cerebrospinal fluid (CSF) and blood following neuroaxonal damage [11]. Independent of MS, recent studies have shown that individuals with major depressive disorder (MDD) have higher blood NfL levels than healthy controls, and meta-analytic evidence suggests NfL may help distinguish depressive disorders from non-psychiatric states [12]. Conversely, in MS populations, there is limited data on whether NfL correlates with psychiatric burden (e.g., depression, anxiety) beyond its established role in reflecting neuroaxonal injury [13]. Thus, although NfL is validated as a marker of axonal damage in MS and shows promise in psychiatric research, the relationship between NfL levels and psychiatric symptoms in MS remains insufficiently characterized [14]. This gap is especially important in settings like Iraq, where MS prevalence is rising [2], psychiatric comorbidity may be underdiagnosed, and resources for psychiatric evaluation are constrained. The rationale for this study is to clarify whether serum NfL can serve not only as a biomarker of neurological damage in MS but also correlate with psychiatric burden, specifically anxiety and depression, in this population. Demonstrating such associations could support earlier detection and more integrated care of psychiatric symptoms, possibly using NfL as a tool to flag patients needing mental health evaluation. Accordingly, this study aims to evaluate serum neurofilament light chain (sNfL) levels in Iraqi patients with multiple sclerosis and to assess the relationship between sNfL and psychiatric symptoms of anxiety and depression. We hypothesize that while sNfL will correlate strongly with measures of neurological disability, it may or may not correlate with severity of anxiety and depression. The findings will inform whether sNfL has utility beyond neurological monitoring, potentially guiding more comprehensive patient management.

METHODS

Study Design and Setting

This was a cross-sectional, case-control study conducted at the Multiple Sclerosis Consultation Clinic, Iraqi Medical City, Baghdad. The study was carried out under the supervision of a consultant neurologist between the 1st of October 2024 and the 31st of January 2025. A total of 200 participants were recruited: 150 patients with clinically confirmed MS and 50 healthy controls matched by age and sex. Healthy controls were recruited from non-hospital sources (e.g., relatives, private pharmacies) and had no history of neurological, psychiatric, or systemic autoimmune diseases.

Inclusion criteria

Age ≥18 years. Diagnosis of MS according to the revised McDonald criteria for at least one year [15]. Currently receiving disease-modifying therapy for multiple sclerosis, including interferon beta, fingolimod, natalizumab, teriflunomide, rituximab, and ocrelizumab, and in clinical remission at the time of study enrollment. Ability to understand and complete the study questionnaires.

Exclusion criteria

Current or prior diagnosis of depression or anxiety disorder before MS onset. Use of antidepressant or anxiolytic medications. History of stroke, migraine, or other autoimmune or neurological diseases. Pregnancy or lactation. Cognitive impairment, speech difficulties, or severe mental illness may hinder the completion of the questionnaire.

Psychiatric assessment

Anxiety and depression were evaluated using the Hospital Anxiety and Depression Scale (HADS), which is validated in Arabic [16,17] (Table 1).

Table 1: Hospital Anxiety and Depression Scale (HADS) for multiple sclerosis (MS) and control groups

Groups		MS	Control	*p*-value*
HADS-D	Mild (0-7)	80(53.33)	47(94)	
	Moderate (8-10)	23(15.33)	1(2)	<0.001
	Severe (11-21)	47(31.33)	2(4)	
HADS-A	Mild (0-7)	25(16.6)	38(76)	
	Moderate (8-10)	103(68.67)	7(14)	<0.001
	Severe (11-21)	22(14.67)	5(10	

**p*-value by Chi Square test. *p*-value of <0.05 is considered significant difference. Abbreviations: HADS-D: Hospital Anxiety and Depression Scale depression; HASD-A: Hospital Anxiety and Depression Scale anxiety; MS: Multiple Sclerosis

The scale comprises 14 items (7 for anxiety, 7 for depression), each scored from 0 to 3. Subscale scores of 0–7 indicate normal, 8–10 borderline, and ≥11 suggest clinically significant symptoms. This tool has been validated in MS cohorts and is considered appropriate

for distinguishing psychiatric burden in the presence of overlapping somatic symptoms.

Disability assessment

Neurological disability was assessed using the Expanded Disability Status Scale (EDSS), administered by a neurologist. The EDSS ranges from 0 (normal neurological examination) to 10 (death due to MS), capturing both ambulation and functional system impairments [18]. The questionnaires were primarily completed by the participants themselves. Participants with visual impairments or limited literacy skills received assistance from the researcher, and the responses were recorded exactly as the participants stated them in order to minimize bias during data collection.

Biomarker measurement

Serum NfL concentrations were quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) (Human NEFL ELISA kit, Reed Biotech, catalog no. RE1410H). All assays were performed in duplicate following the manufacturer's protocol. Results were expressed in picograms per milliliter (pg/mL). Internal standards and blanks were included for quality control.

Sample size justification

Sample size estimation was performed using power analysis for correlation. For a two-tailed test at $\alpha = 0.05$, power $(1-\beta) = 0.80$, and an expected correlation of $r = 0.30$ (moderate effect size according to Cohen [19, 20]), the minimum required sample was calculated using the formula:

$$n = \left(\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{0.5 \times \ln\left(\frac{1+r}{1-r}\right)}\right)^2 + 3$$

Where: $Z_{1-\alpha/2} = 1.96$ (for $\alpha = 0.05$, two-tailed); $Z_{1-\beta} = 0.84$ (for 80% power); and $r = 0.30$.

Substituting values yields a minimum sample size of $n \approx 85$ patients. To increase robustness, account for potential missing data, and enable subgroup analyses (e.g., stratification by Expanded Disability Status Scale [EDSS] score or psychiatric symptom severity), the recruitment target was expanded to 150 patients with MS. In addition, 50 age- and sex-matched controls were included to provide normative reference values for serum neurofilament light chain (sNfL).

Bias

Potential confounding arising from demographic variables was minimized by recruiting a healthy control group matched for age and sex. Selection bias was mitigated through the use of a case-control design with strict inclusion and exclusion criteria. Specifically, participants with pre-existing psychiatric disorders or those using psychotropic medications were excluded to avoid confounding the relationship between MS pathology and current psychiatric symptoms. To control for demographic confounders, the control group was matched to the patient group by age and sex. Measurement bias was addressed by using the linguistically validated Arabic version of the Hospital Anxiety and Depression Scale (HADS) to ensure cultural applicability, while sNfL levels were quantified using a standardized ELISA protocol with duplicates to ensure assay precision. Subjective psychiatric burden was evaluated using the HADS, a tool previously validated for sensitivity in distinguishing psychiatric symptoms from somatic MS symptoms. Sample size adequacy was determined via a priori power analysis to ensure sufficient statistical power to detect moderate effect sizes. Finally, to reduce the risk of Type I errors during statistical analysis, a Bonferroni correction was applied to multiple group comparisons.

Ethical considerations

The study protocol was approved by the Ethics Committee of the College of Pharmacy, Mustansiriyah University. Written informed consent was obtained from all participants prior to enrollment. Data confidentiality and anonymity were maintained in accordance with the Declaration of Helsinki.

Statistical analysis

Data were analyzed using IBM SPSS Statistics v25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were tested for normality using the Shapiro–Wilk test. As distributions were not normally distributed, results are presented as median (range). Mean ± standard deviation was reported for descriptive purposes only where appropriate. Group comparisons: Mann–Whitney U test or Kruskal–Wallis test with Bonferroni correction. Categorical variables: Chi-square test or Fisher's exact test as applicable. Correlations: Spearman's rank correlation coefficient to evaluate associations between sNfL, EDSS, and HADS scores. A two-sided $p < 0.05$ was considered statistically significant.

RESULTS

A total of 200 participants were enrolled, comprising 150 patients with MS (58% female, mean age 35.1 ± 11.0 years) and 50 healthy controls (70% female, mean age 29.1 ± 10.1 years). Among MS patients, the median disease duration was 8 years (range: 1–42 years), and relapsing–remitting MS was the predominant subtype (86.0%). MS patients demonstrated significantly higher Hospital Anxiety and Depression Scale scores compared

with controls. The median Hospital Anxiety and Depression Scale–Depression subscale (HADS-D) score was 7 (range: 0–21) in MS patients versus 3 (0–15) in controls ($p< 0.001$). Similarly, median Hospital Anxiety and Depression Scale–Anxiety subscale (HADS-A) scores were 4 (0–19) versus 2 (0–20), respectively ($p< 0.001$). Overall, 31.3% of patients met criteria for depression and 14.7% for anxiety, compared with 4.0% and 10.0% among controls. Depression among patients with multiple sclerosis was strongly associated with socioeconomic and educational status. Higher median HADS-D scores were seen in patients with income 1M (4 [0–15]; $p< 0.001$), as seen in Table 2.

Table 2: Association of HADS -D and HADS-A scores with different patients and disease characteristics

Variable		HSDS-D	*p*-value	HADS-A	*p*-value
Age	18-29	4(0-15) [a]		4(0-17)	
	30-39	6(0-21) [a-b]	0.001	5(0-17)	0.514
	40-50	6(0-20)		3(0-19)	
	>50	11(0-20)		3(0-20)	
SEX	Female	5(0-20)	0.702	5(0-20)	<0.001
	Male	6(0-21)		3(0-16)	
BMI	Underweight	6(3-13)		2(1-11)	
	Normal	6(0-21)		4(0-19)	
	Overweight	4(0-19)	0.366	4(0-20)	0.104
	Obese class I	5(0-20)		3(0-17)	
	Obese class II	6(1-8)		2(0-10)	
	Obese class III	15(6-20)		17(10-18)	
Marital status	Married	6(0-20) [a-b]		4(0-20)	
	Single	4(0-21) [a]	0.004	3(0-17) [a]	0.026
	Divorced	8(2-15) [a-c]		7(2-17) [a-c]	
Economic state	<500K	9(0-20) [a-b]		5(0-19)	
	500K-1M	5(0-21) [a-c]	<0.001	4(0-17)	0.271
	>1M	4(0-15) [a]		2(0-20)	
Educational level	Illiterate	12(6-19) [a-d]		7(2-18)	
	Primary	9(1-20) [a-c]		7(0-17)	
	Secondary	6(0-20) [a-b]	<0.001	4(0-19)	0.072
	Collage	4(0-19) [a]		3(0-20)	
	Higher education	6(0-21)		3(0-13)	
Residency	Urban	5(0-21)	0.286	4(0-20)	0.643
	Rural	7(0-20)		3(0-18)	
Family history	No	7(0-21)	0.802	4(0-19)	0.385
	Yes	8(1-11)		4(0-10)	
Drinking	No	6(0-21)	0.197	4(0-20)	0.913
	Yes	4(0-12)		4(0-16)	
Smoking	No	5(0-21)	0.134	4(0-20)	0.026
	Yes	8(0-20)		3(0-14)	
comorbidity	No	5(0-21)	0.444	4(0-19)	0.672
	Yes	6(0-20)		3(0-20)	
Disease duration	1-5	6(0-21)	0.328	4(0-17)	0.708
	>5	7(0-20)		4(0-19)	
Type of MS	RRMS	7(0-19)		4(0-19)	
	PPMS	9(1-21)	0.191	8(0-14)	0.235
	SPMS	11(2-20)		5(1-17)	
Treatment	interferon beta-1a (Avonex)	7(5-10)		3(1-8)	
	interferon beta-1a (Rebif)	6(1-18)		4(1-9)	
	interferon beta-1b	6(0-15)		4(1-18)	
	Teriflunomide	9(1-16)	0.074	3(0-5)	0.833
	Natalizumab	6(0-19)		4(0-19)	
	Fingolimod	10(3-14)		4(0-17)	
	Rituximab	9(0-20)		6(0-17)	
	Ocrelizumab	16(2-21)		5(1-14)	
Treatment duration cat	<1	12(0-20)		10(0-17)	
	1-<2	7(1-19)	0.218	4(0-10)	0.080
	2-5 Y	7(0-21)		5(0-17)	
	>5 y	6(0-16)		3(0-19)	
Last MS relapses	No relapse	7(0-21)		4(0-17)	
	1-2 Y	7(0-19)		5(0-18)	
	>2 -<3 Y	7(0-20)	0.160	4(0-17)	0.124
	3-5 Y	10(0-20)		7(1-17)	
	>5 Y	6(0-15)		3(1-19)	

Values are expressed as median and range. *p*-value using Mann Whitney test or Kruskal Wallis test as appropriate. Superscript letters represented Bonferroni post hoc test, a,c indicated significant difference after adjustment for multiple testing, black color (a,b,c) denoted not maintained significant after adjustment for multiple testing. P-value of <0.05 is considered significant difference, while >0.05 is considered not significant difference. Abbreviation: HADS-D: Hospital Anxiety and Depression Scale depression; HASD-A: Hospital Anxiety and Depression Scale anxiety; MS: Multiple Sclerosis; RRMS: relapsing-remitting multiple sclerosis, PPMS: primary progressive multiple sclerosis, SPMS: secondary progressive multiple sclerosis, BMI: body mass index; K: thousands Iraqi dinar, M: million Iraqi dinar.

Likewise, the depressiveness scores were highest in illiterate patients (12 [6–19]) compared to patients with higher education (6 [0–21]) ($p< 0.001$). Associations with younger patients (<30 years) (OR= 0.97; 95% CI 0.95–0.99) and single individuals (OR= 0.95; 95% CI 0.93–0.97) had the lowest score for depression (4 [0–15] and 4 [0–21], respectively), but lost significance after adjustment. In contrast, demographic and lifestyle factors had stronger associations with symptoms of anxiety. Compared to females, males had significantly lower HADS-A scores (3 [0–16]) compared to females (5 [0–20]; $p< 0.001$), and HADS-A was also significantly lower in single (3 [0–17]) than in married (4 [0–20]) or divorced individuals (7 [2–17]; $p= 0.026$).

Smokers (3 [0–14]) had less anxiety than non-smokers (4 [0–20]) (p= 0.026). Patients with lower education levels also tended to be more anxious compared to those with higher education (illiterate: 7 [2–18] vs. higher education: 3 [0–13]; p= 0.072). Other clinical variables, including BMI, comorbidities, treatment duration, and time since last relapse, showed no significant associations. Patients with SPMS had higher depression (11 [2–20]) than those with RRMS (7 [0–19]), and Ocrevus users recorded the highest depressive median (16 [2–21]), but these differences were not statistically significant as presented in Table 2. The median serum neurofilament light chain (sNfL) level in the MS group was 169.6 pg/mL (range: 102.8–345.9), which was significantly higher than that of the control group (98.9 pg/mL, 64.4–145.1; p< 0.001) (Figure 1). Within the MS cohort, sNfL levels varied across Hospital Anxiety and Depression Scale (HADS) categories. For depression, patients with borderline scores (8–10) had the highest median sNfL (177.1 pg/mL), compared with those with normal scores (0–7; 177.6 pg/mL) and case-level depression (11–21; 153.5 pg/mL), yielding a significant difference (p= 0.002). For anxiety, patients with borderline scores (8–10) showed elevated sNfL (177.8 pg/mL) relative to those with no anxiety (0–7; 155.5 pg/mL) or case-level anxiety (11–21; 163.9 pg/mL) (p= 0.012). However, these associations did not remain significant after correction for multiple testing (Table 3).

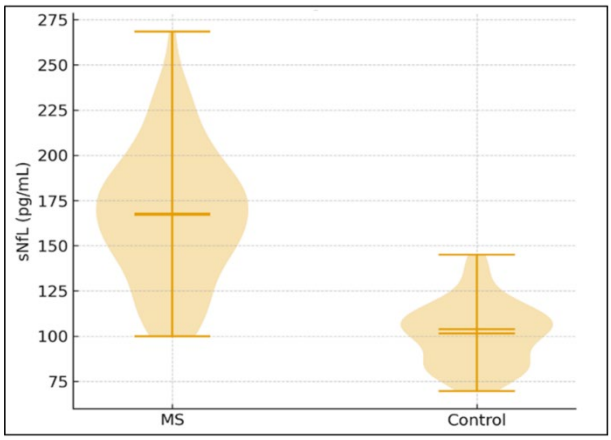

Figure 1: Comparison of neurofilament level between multiple sclerosis group (MS) and control. The difference is significant (Mann Whitney test: p<0.001).

Table 3: comparison of sNfL level between MS and control groups according to different demographic and socioeconomic parameters

Variable		MS	*p*-value	Control	*p*-value
Age	18-29	176.1(102.8-341.9)		98.97(64.4-140.4)	
	30-39	164.15(127.98-345.96)	0.174	104.6(73.5-145.1)	0.881
	40-50	176.1(131.3-245.2)		110.6(84.2-144.9)	
	>50	149.5(131.5-237.8)		93.1(82.9-103.2)	
Sex	Female	172.2(102.8-345.96)	0.785	98.7(64.4-145.1)	0.866
	Male	167.15(131.3-341.9)		103.2(73.5-144.9)	
BMI	Underweight	199.16(140.5-204.9)		-	
	Normal	165.66(102.8-308.2)		97.5(64.4-145.1)	
	Overweight	165.6(110.3-341.9)	0.732	110.2(82.9-143.3)	0.098
	Obese class I	188.7(130.7-324.1)		87.6(73.5-113.5)	
	Obese class II	246.6(147.2-345.96)		139.1(133.3-144.9)	
	Obese class III	200(172.2-214.8)		-	
Marital status	Married	172.7(107.6-345.96)		93.(64.-125.)	
	Single	169.8(102.8-295.2)	0.198	105.5(73.5-145.1)	0.279
	Divorced	157.8(126.6-218.1)		-	
Economic state	<500K	161.6(110.3-283.95)		93.1(73.5-136.0)	
	500K-1M	177.2(102.8-345.96)	0.096	105.5(64.4-145.1)	0.086
	>1M	164.5(135.4-185.4)		103.2(82.9-143.1)	
Educational level	Illiterate	146.8(127.98-237.0)		-	
	Primary	172.7(107.6-283.95)		-	
	Secondary	162.3(102.79-308.2)	0.374	125.8(73.5-144.9)	0.867
	Collage	177.18(110.3-345.96)		98.7(64.4-145.1)	
	Higher education	165.3(134.8-324.1)		113.6(84.2-143.1)	
Drinking	No	169.6(102.8-345.96)	0.877	98.7(64.4-145.1)	0.516
	Yes	182.9(147.8-218.1)		113.5(92.6-140.4)	
Smoking	No	171.9(102.8-345.96)	0.543	98.72(64.4-145.1)	0.800
	Yes	163.49(110.3-341.9)		113.5(113.5-113.5)	
Comorbidity	No	174.8(102.8-345.963)	0.016	98.98(64.4-145.1)	0.982
	Yes	154.08(137.8-197.95)		108.6(73.5-144.9)	
HADS-D	0-7	177.59(102.79-341.91) [a-b]		98.98(64.4-145.1)	
	8-10	177.13(131.3-345.96) [a-c]	0.002	-	-
	11-21	153.5(126.6-282.5) [a]		-	
HADS-A	0-7	155.5(131.3-230.4) [a]		100.8(64.4-145.1)	
	8-10	177.8(107.6-345.96) [a-b]	0.012	97.9(79.5-113.5)	0.596
	11-21	163.95(102.8-308.2)		119.6(98.7-140.4)	

Values are presented as median and range. *p*-value by Mann Whitney test or Kruskal Wallis test as appropriate. Superscript letters represented Bonferroni post hoc test, a,b,c indicated lost significant difference after adjustment for multiple testing. *p*-value of <0.05 is considered significant difference. Abbreviations: MS: Multiple Sclerosis, RRMS: relapsing-remitting multiple sclerosis, PPMS: primary progressive multiple sclerosis, SPMS: secondary progressive multiple sclerosis, BMI: body mass index, K: thousands Iraqi dinar, M: million Iraqi dinar, HADS-D: Hospital Anxiety and Depression Scale depression, HASD-A: Hospital Anxiety and Depression Scale anxiety.

Analyses stratified by demographic and socioeconomic variables revealed no significant associations between sNfL and age, sex, body mass index, marital status, economic state, education level, alcohol consumption, or smoking (p > 0.05 for all). The only significant difference was observed for comorbidity: patients without comorbid conditions had higher median sNfL (174.8 pg/mL, 102.8–345.9) compared with those with comorbidities (154.1 pg/mL, 137.8–198.0; p= 0.016). It was found that the levels of sNfL in the blood had a

weak but statistically significant negative relationship with HADS-D depression scores (r= –0.174, *p*= 0.034). On the other hand, there were no significant links found between sNfL levels and either HADS-A scores or EDSS scores (*p*> 0.05). There was a weak but significant link between EDSS scores and age (r = 0.193, p = 0.018), depression (HADS-D: r= 0.400, *p*< 0.001), and anxiety (HADS-A: r= 0.244, *p*= 0.003). This suggests that the burden of disability rises with both older age and more severe mental symptoms. Also, HADS-D scores were linked to age in a positive way (r= 0.297, *p*< 0.001), which means that older MS patients are more likely to have depressed symptoms. Overall, these results show that sNfL doesn't consistently track mental symptoms or disability. However, it is clear that depression and disability rise with age, highlighting how the clinical and behavioral burdens of MS are connected, as presented in Figure 2 and Table 4.

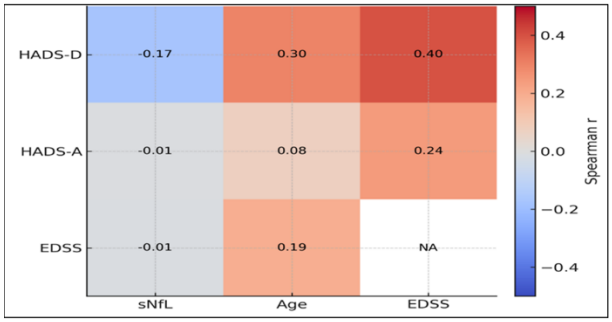

Figure 2: Heatmap of Spearman correlations among sNfL, Age, EDSS, and HADS Scores in Multiple Sclerosis.

Table 4: Correlations between sNfL, age, EDSS and HADS scores in MS patients

Variables	sNFL		AGE		EDSS	
	r	*p*-value	r	*p*-value	r	*p*-value
HADS- D	-0.174	0.034	0.297	<0.001	0.4	<0.001
HADS -A	-0.010	0.905	0.076	0.355	0.244	0.003
EDSS	-0.014	0.862	0.193	0.018		

Spearman linear correlation. *p*-value of <0.05 is considered significant. Abbreviations: HADS-D: Hospital Anxiety and Depression Scale depression, HASD-A: Hospital Anxiety and Depression Scale anxiety. EDSS: Expanded Disability Status Scale, sNfL: serum neurofilament light chain.

DISCUSSION

Findings of this study showed that approximately 31.3% of MS patients had depression and 14.7% had anxiety symptoms, significantly exceeding rates in controls. These depression figures align with global pooled estimates (e.g., 30.5% for depression in MS and 22.1% for anxiety) from meta-analyses [7]. In a recent large MS cohort, the prevalence of depression was estimated at 25.3% [21], somewhat lower than ours, possibly reflecting methodological or population differences. Meanwhile, anxiety prevalence in MS has been reported at ~36% in a systematic review [22], markedly higher than our finding of 14.7%, which suggests variation by region, cultural factors, or measuring instruments [6]. A Saudi study reported depression in ~42.7% and anxiety in 26% of MS patients [23]. Our findings reinforce that depression is more prevalent than anxiety among MS patients, as a Saudi study reported depression in ~42.7% and anxiety in 26% of MS patients, a trend also reported in studies from Saudi Arabia [24,25]. In Iraq specifically, comprehensive population data on psychiatric comorbidity in MS remain sparse; a previous study showed that MS patients had a 26.8% risk of depression [26], while another previous MS epidemiological study in Iraq emphasized rising incidence and burden but did not quantify psychiatric symptoms extensively [2]. Thus, our data provide an important addition to the Iraqi literature, demonstrating that psychiatric comorbidity is substantial even in this setting [10]. We discovered that lower socioeconomic status (SES) and educational attainment were strongly linked with greater depression ratings in our MS sample. Higher anxiety scores were significantly associated with female sex and divorce status. As with other socioeconomic determinants of mental health, lower SES and education are risk factors for depression in many societies [26,27]. Lower SES may increase stress, restrict access to psychological support and health services, and diminish MS patients' resistance to chronic illness. Similar relationships were seen in previous investigations; a nationwide screening found that female gender, poor income, and low education predicted depression and anxiety in the general population [28]. Lower levels of education are often linked to poorer mental health outcomes. In Iraq, the lack of educational opportunities due to prolonged conflict and instability has contributed to a population with limited mental health literacy, which can exacerbate mental health issues [29]. An Iraqi study reported higher depression rates among lower-income patients [30]. In our findings, we observed higher depressive scores among older patients, as reported previously by Sulaiman *et al*. [31]. This may be explained by reduced physical activity or impairments in cognitive and social capacity. Female sex and divorce status were significantly associated with higher anxiety scores. Our finding that female sex and marital status affect anxiety is confirmed by MS and general psychiatric literature. Women have a greater incidence of mood and anxiety disorders globally, perhaps related to hormonal changes, caregiving, and societal stresses [32]. Marital status is more complicated: some studies show that social isolation (single, divorced, widowed) increases depression/anxiety, but in some cultures, marriage can also cause stress, caregiving, or interpersonal strain, which may increase anxiety [33,34]. Our conclusion that marital status is associated with increased anxiety may reflect social norms and expectations in our group. Interestingly, we observed lower anxiety levels among smokers and single individuals, which contrasts with most literature [35,36]. Typically, smoking is associated with greater psychiatric symptomatology [37]. It is possible that in our sample, smokers represent a subgroup with different

coping styles or unmeasured confounders, or that a single individual's context has fewer social pressures [38]. These findings should be interpreted cautiously and warrant further study [39]. We found that sNfL was significantly elevated in MS patients relative to controls, confirming its role as a biomarker of neuroaxonal injury [40]. However, associations between sNfL and psychiatric symptoms were weak or non-significant: borderline depression or anxiety groups had marginally higher sNfL than severe groups, and correlation analysis revealed a weak negative association between sNfL and depression ($r = –0.174$). In previous MS cohorts no association between sNfL and indicators of depression, anxiety, fatigue, or quality of life was found, particularly not in stable and clinically remitted patients [41]. In exploratory studies in psychiatric patients with presumed neuroinflammation, NfL was more elevated than expected but not relative to symptom severity [42]. These data underpin the notion that sNfL is a biomarker of axonal loss but not of psychological morbidity in MS. None of the demographic variables showed significant associations with sNfL levels; however, the presence of comorbidities was associated with slightly higher sNfL concentrations compared with patients without comorbidities. This pattern may reflect more recent or insufficiently treated inflammatory activity in these individuals, whereas patients without comorbidities may represent clinically stable cases receiving disease-modifying therapies (DMTs) that are known to lower sNfL levels. Furthermore, sNfL was not associated with disability level (EDSS), a finding consistent with other reports [43]. In fact, an additional large longitudinal study discovered that sNfL concentrations increase with age, are higher in women, and correlate with more disability, yet do not correlate significantly with depressive symptoms [44]. We documented significant positive correlations between disability (EDSS), age, and severity of depressive and anxiety symptoms. Older patients and those with more advanced disabilities had a greater psychiatric burden. These trends align with global studies: greater disability is a consistent risk factor for depression and anxiety in MS [45]. In general, our findings are consistent with trends seen in other countries and regions, particularly in terms of higher rates of depression and links to sociodemographic and clinical factors [46]. Differences in anxiety prevalence or the weak linkage between sNfL and psychiatric symptoms might reflect local sociocultural factors, measurement differences, or sample-specific issues [47], which could impact the generalizability of our findings and suggest the need for further research to explore these variables in different contexts.

Study limitations

There are some limitations in this study to declare; First, the cross-sectional design limits the ability to draw causal conclusions about the association between psychiatric symptoms, disability, and serum neurofilament light chain (sNfL) levels. We would need longitudinal follow-up to clarify temporal and possibly predictive associations. Second, the psychiatric comorbidities were evaluated by the Hospital Anxiety and Depression Scale. Although this scale is validated in MS populations, it is still a self-report instrument and may misclassify symptoms because of overlap between the two conditions; for example, fatigue or cognitive slowing is common in patients with MS and may misclassify them as patients with depression. Third, the sample size, though relatively large for a single-center Iraqi study, may have limited power for subgroup analyses, especially when stratifying by sociodemographic factors, treatment categories, or disease subtypes. Fourth, all patients were recruited in clinical remission and on disease-modifying therapies that might reduce the expression or severity of psychiatric symptoms compared with patients who were in a relapsing state or untreated cohorts. Fifth, the lack of neuroimaging data (e.g., MRI lesion load or volumetric brain atrophy measures) limited investigation of structural correlates that may have offered mechanistic insights into the neurodegeneration-sNfL-psychiatric outcome angle. Finally, the study population came from a single center in a tertiary referral center in Iraq and may be less generalizable to the community or rural-based MS populations with differing access to care and psychosocial support.

Conclusion

Prevalence of depression and anxiety was high among multiple sclerosis patients in this Iraqi cohort and their relationships with age and disability. In MS, and relative to controls, sNfL levels were increased, and as reported previously, sNfL demonstrated its utility as a biomarker of axonal injury; however, associations of sNfL with psychiatric symptoms were weak and inconsistent. These results indicate that, although NfL is a sensitive biomarker of neurotoxicity, it may not replace direct psychiatric evaluation of patients. Integrated care models that address both neurological and mental health aspects of MS are an important step to optimize MS patient outcomes, as they can provide comprehensive treatment that considers the interplay between physical and mental health challenges faced by patients.

Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data can be viewed in Zenodo (Demographic details, clinical data as well as questionnaire responses. https://doi.org/10.5281/zenodo.17936424).

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