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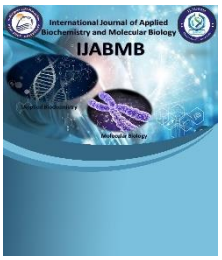

Role of Neurofilament Light Chain in Relapsing Remitting Multiple Sclerosis Patients

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Abstract:

Background: Neurofilament light chain (NfL) is released from damaged neurons and elevated levels in blood reflect ongoing neuroaxonal injury in multiple sclerosis (MS). Assessing NfL levels could provide insight into disease activity and treatment responses in MS patients.

Objective: To investigate the relationship of plasma NfL levels with clinical and MRI parameters and disability scores in relapsing-remitting MS (RRMS) patients.

Patient and method: Plasma NfL concentrations were measured by ELISA in 42 RRMS patients in remission and 42 healthy matched controls. Associations with demographics, disease characteristics, MRI lesion counts, and Expanded Disability Status Scale (EDSS) scores were analyzed.

Results: RRMS patients had significantly higher NfL levels than controls ($p=0.009$). Levels were lower in treated vs untreated patients ($p<0.001$) and correlated positively with age, disease duration, relapse numbers, T2-lesion burden and EDSS scores ($p<0.05$). No correlation was observed with recent relapse rate or treatment duration.

Conclusion: Increased plasma NfL levels were associated with greater disability in RRMS patients and were lowered by treatment, indicating potential neuroprotective effects. NfL could be a useful biomarker in monitoring subclinical disease activity and treatment responses longitudinally.

Keywords: Neurofilament light chain, multiple sclerosis, biomarkers, disability, EDSS.

Introduction:

Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system characterized by myelin damage, axonal loss, and progressive neurological disability. MS is a leading cause of nontraumatic disability among young adults, affecting over 2.8 million people worldwide (1). While several disease-modifying therapies (DMTs) for MS are available, monitoring treatment response and disease progression remains challenging (2).

Neurofilaments (Nf) are important structural proteins located in neurons and axons. Neurofilament light chain (NfL) is a key subunit that is released into the cerebrospinal fluid and blood upon neuroaxonal injury (3). Prior research indicates that blood NfL levels are elevated in MS patients compared to healthy controls, correlate with clinical and MRI outcomes, and decrease with effective DMTs. This suggests the potential utility of serum NfL measurement as a prognostic and treatment response biomarker that may enable personalized therapeutic decisions in MS (4).

However, data on correlations of blood NfL levels particularly with disability assessed by the Expanded Disability Status Scale (EDSS) and MRI lesion parameters in Egyptian MS patients is currently lacking. Additionally, the effects of high-efficacy DMTs, including fingolimod, on NfL levels had not been extensively studied (5).

Therefore, in this study we aimed to evaluate the associations of plasma NfL concentrations with demographic, clinical and MRI characteristics and EDSS scores in Egyptian patients with relapsing-remitting MS (RRMS). We also compared NfL levels among RRMS patients on DMTs, untreated patients, and healthy matched controls.

Patients and Methods:

This case-control study was conducted on 42 relapsing-remitting MS (RRMS) patients diagnosed based on the 2017 McDonald criteria (6) and 42 matched healthy controls. Consecutive stable RRMS patients followed at the Multiple Sclerosis clinic of Beni-Suef University Hospital, Egypt were recruited over a one-year period between January 2022 and January 2023. Controls without any neurological disease were selected from the same geographic area.

Plasma samples were collected from RRMS patients and controls. Levels of neurofilament light chain (NfL) were measured using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol. OD (Optical densities) were read using an automated microplate reader and NfL concentrations were derived from standard curves. Information on patient demographics, clinical characteristics and MRI features were retrieved from medical records using a standardized data collection form. Disability was scored using the Expanded Disability Status Scale (EDSS).

Approvals were obtained from the institutional research ethics committee prior to study commencement. Written informed consents were taken from all enrolled participants (Approval No: FMBSUREC/05122021/Mohamed).

Statistical analysis was performed using SPSS version 26. Normality of data was assessed using the Shapiro-Wilk test. Between group differences in plasma NfL levels were compared by independent samples t-test. Correlations of NfL concentrations with quantitative variables were examined by bivariate Pearson's correlation. P-values <0.05 were considered statistically significant.

The STROBE guidelines for reporting observational studies were followed in this study. Sample size was calculated to provide 80% power to detect a moderate effect

size of 0.8 between RRMS patients and controls in mean NfL levels at 5% significance level.

Results:

The current study was conducted at Beni-Suef university hospital over a one-year period between January 2022 and January 2023, the study included 42 RRMS patients and 42 matched healthy controls.

Table 1 Comparison of NFL plasma level (ng/l) in different study groups

Group	NFL plasma level (ng/l)				
	N	Mean	SD	P-value	Sig.
Cases	42	35.87	8.6	0.009	S
Controls	42	31.92	4.1		

Table 2 Comparisons of NFL plasma level (ng/l) between treated & untreated cases

Group	NFL plasma level (ng/l)				
	N	Mean	SD	P-value	Sig
Untreated cases	13	42.99	7.5	<0.001	S
Treated cases	29	32.67	7.1		

Table 3 Comparison of NFL plasma level (ng/l) between Treated cases & control

Group	NFL plasma level (ng/l)				
	N	Mean	SD	P-value	sig
Treated cases	29	32.67	7.1	0.6	NS
controls	42	31.92	4.1		

Table 4 Comparison of NFL plasma level (ng/l) between Untreated cases & control

Group	NFL plasma level (ng/l)				
	N	Mean	SD	P-value	sig

Untreated cases	13	42.99	7.5	<0.001	S
Controls	42	31.92	4.1		

The head-to-head comparisons of plasma NfL concentrations elucidate valuable differences between RRMS cases and controls as well as treated and untreated patient strata. The markedly higher levels in RRMS patients versus controls reflects ongoing neurodegeneration. However, the lack of significant difference between DMT-treated cases and controls indicates these drugs may impart neuroprotective benefits and reduce neuronal damage in MS.

The much higher NfL levels again among non-treated patients signifies uncontrolled disease activity and progressive neuroaxonal injury in absence of effective therapy.

Table 5 Correlation between NFL plasma level with Clinical and Radiological variables among MS group

Clinical Variables	**NFL plasma level**		
	r	**P-value**	**Sig.**
Age (years)	0.5	0.001	S
Disease duration (yrs)	0.39	0.01	S
Age of disease onset (yrs)	0.29	0.06	NS
Total number of relapses	0.48	0.001	S
Number of relapses in last 2 years	0.05	0.76	NS
Duration of Current MS treatment	0.02	0.9	NS
EDSS	0.65	<0.001	S
Radiological Variables			
Number of supra tentorial T2 lesions	0.5	0.001	S

Number of infra tentorial & spinal cord T2 lesions	0.4	0.006	S
Total number of T2 lesions	0.5	0.001	S

The positive correlations demonstrate important relationships of plasma NfL levels with parameters that may determine or reflect MS disease severity like age, disease duration, clinical relapse events, MRI lesion load and physical disability measured using the EDSS scale.

Higher NfL levels tracked with greater age, disease duration, relapse numbers, lesion burden and worsening disability scores - thereby validating the potential use of this biomarker as an indicator of the magnitude of neuronal injury and associated clinical worsening in RRMS patients.

Discussion:

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system that is mediated by the immune system. It is characterized by the presence of inflammation, demyelination, and peeling of axons.MS generally manifests in individuals in the young adult population, with an average age of onset ranging from 20 to 30 years. This condition can result in physical disability, cognitive dysfunction, and a reduction in overall quality of life (7).

Neurofilament Light chain (NfL), a neuron-specific cytoskeletal protein produced into extracellular fluid following axonal damage, has been noticed as a biomarker of disease activity in MS (8).

An increasing body of scientific research has demonstrated that the presence of neurofilament light chain (NfL) in both cerebrospinal fluid (CSF) and serum can serve as dependable markers for assessing prognosis and measuring the effectiveness of treatment. In recent times, the utilization of NfL has demonstrated its potential in aiding

the process of making personalized treatment choices for individuals diagnosed with MS (9). Yet, there is lack of research that has explored the effects of high-efficacy disease-modifying therapy (DMT) on levels of blood NfL (10).

Our results revealed that there was statistically significant difference in NFL plasma level (p-value=0.009) between cases and controls (Cases were higher). Similar findings were obtained by Kuhle (11) who found that, at baseline, patients had significantly higher blood NfL concentrations than healthy controls. Disanto (3) also declared that patients had higher serum NFL levels than healthy controls.

Our results revealed that there was statistically significant difference in NFL plasma level with p-value <0.001 between untreated and treated cases. Mean NFL plasma level in untreated cases was 42.99 ng/l while Mean NFL plasma level in treated cases was 32.67 ng/l. Also, Bridel (12) found significantly higher serum NFL in untreated relapsing remitting multiple sclerosis patients than treated patients. In contrast to our findings, Barro (13) noted that NFL levels were not lower in treated patients compared to untreated individuals in this randomized controlled trial.

Patients in Barro`s results (13) were with a low mean disease duration are typically treated, unless their disease has a mild activity that corresponds to lower sNFL levels; thus, there is a nonrandom assignment of treatment and a selection bias. Secondly, they evaluated a patient population at an advanced disease stage where treatments are the least effective and therefore have the least potential for decreasing sNFL levels.

Our results revealed that there was no statistically significant difference in NFL plasma level with p-value 0.6 between Treated cases and controls.

According to our findings, Sejbaek (14) who examined NFL levels by single- molecule array in 88 CSF, 348 plasma and 131 sera from treatment- naïve RRMS patients, healthy controls and a placebo group matched by age, sex. Plasma/sera were collected at baseline, and 1, 3, 6 and 12 months after DMF (Dimethyl fumarate). They found that

NFL concentration in the plasma of MS patients became like that of healthy controls after 6- and 12-months of treatment.

The previous result could be simply explained by treatment effect on lowering NFL levels to approach those levels of healthy individuals, which supports the rising value of NFL in monitoring treatment response and measuring efficacy of DMT in MS patients.

In our study, there was statistically significant difference in NFL plasma level with p-value <0.001 between Untreated cases and controls. Accordingly, Hakansson (15) compared NFL levels in health and disease. Patients at baseline (all untreated) were compared with healthy controls. They found that serum NFL was significantly higher in patients than in controls.

There was a positive significant correlation between MS patients' age and plasma NFL level. In accordance with our findings, Disanto used univariate and multivariate Models Testing Associations between Age and serum NFL. They found significant correlation of NfL concentrations and age in all MS patients included in the study with p-value <0.001 (16). The observed increase of NfL levels in serum with age seen in patients is best explained by ongoing age-related neuronal degeneration (3).

There was a positive significant correlation between disease duration with plasma NFL level in MS patients. Also, there was significant correlation between plasma NFL level and disease duration in MS patients included in FREEDOMS trial (11).

There was a positive significant correlation between the total number of relapses with plasma NFL level in MS patients. Wendel (17) reached similar result as they found that serum NfL levels were significantly associated with increased number of relapses.

There was statistically significant positive correlation with p-value <0.05 between NFL plasma level and each of number of supra tentorial T2 lesions, number of infra tentorial & spinal cord T2 lesions, total number of T2 lesions. Also, Wenger (18) found strong

association between the number of T2 lesions and serum NfL ($r = 0.4$, $p < 0.01$). In contrast to our results, One-hundred-twenty-nine patients (111 RRMS and 18 PPMS) were recruited in a study that found no correlation between Serum NfL and any of the MRI measurements (19). The observed discrepancies are highlighting the necessity for larger study populations to establish precise correlations between NFL and MRI measurements. The manual counting of MRI lesions is not the most precise method for quantifying the extent of demyelinating lesions. However, it is easiest to use in clinical settings. One more thing to consider is that the sample could not be stratified and the effect of immunomodulatory treatment on NFL levels was not possible to control.

There was a positive significant correlation between EDSS and NFL level. In accordance with our findings, Barro (13) found that the levels of sNFL were positively correlated with EDSS scores.

In contrast to our findings, a study in 2018 found a non-significant correlation between serum NFL and EDSS in 42 RRMS patients that were enrolled in this study (20). The absence of correlation between NFL and EDSS in last mentioned study could be probably attributed to the relative benign disease severity of cases since most of the patients had RRMS with low EDSS (median value is 2).

However, findings are mixed across NfL studies with some showing no significant correlations, highlighting complexities linking biomarker levels with clinical outcome measures (5), (13), (19), (20).

In conclusion, there is accruing support for serum NfL as a prognostic tool in MS. Additional research is warranted elucidating its relationships to clinical course and treatment responses over heterogeneous MS phenotypes and stages. More longitudinal controlled trials can clarify NfL patterns over the full disease duration. Development of clinically meaningful NfL thresholds and integration into future patient management

algorithms hold promise to enable personalized medicine approaches for this complex disease.

Conclusion and Limitations:

NfL can serve as a biomarker reflecting axonal damage and disability progression in RRMS. Treatment reduced NfL levels suggesting a neuroprotective effect. The present study is not devoid of limitations: The study did not include MRI lesions with gadolinium enhancement, only a single standardized high-resolution MRI scan was available as part of the clinical diagnostic workup and no lesion volume measurements were available in addition to the T2 lesion counts to test for association with serum NfL. The study was limited to RRMS patients, further studies should include other types.

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