Serum neurofilament level as a biomarker in multiple sclerosis

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Abstract

Background & Objective: Currently, MS disease activity and neurodegeneration are assessed mainly through clinical evaluation and magnetic resonance imaging. These measures lack sensitivity and specificity, leading to a constant search for a new biomarker. Several markers have been studied, and the most promising to date is the neurofilament. Our objective was to examine the relationship between serum neurofilament levels and multiple sclerosis parameters.

Methods: Fifty-six adults who fulfilled the McDonald’s criteria 2017 for the diagnosis of MS, and forty-four healthy controls were enrolled in this study. Serum samples were collected to assess neurofilament light and heavy chain (NfL & NfH) levels using the ELISA method, the results were compared for MS patients and controls; and were also correlated to the type of MS, disease duration, EDSS (expanded disability status scale), MSSS (MS severity score) and disease activity.

Results: The mean serum NfL and NfH levels for cases were 133.3 pg/ml and 3654.5 pg/ml respectively, which are significantly higher than that for controls (NfL = 80 pg/ml; and NfH = 408.8 pg/ml; p-value < 0.001). There were no significant correlations between serum neurofilament levels and EDSS, MSSS, disease activity, type, and duration.

Conclusions: In our study, we could not find a significant role for the ELISA neurofilament serum level as a biomarker for MS disease activity and severity.

Keywords: Multiple sclerosis, neurofilament light chain, neurofilament heavy chain, serum, ELISA, disability, biomarker.

INTRODUCTION

Multiple sclerosis (MS) is a chronic disease characterized by inflammation and demyelination of the central nervous system (CNS) associated with variable degrees of axonal and neuronal damage. Although demyelination is the hallmark of MS, axonal injury is present, even from the earliest stages of the disease, and appears to be an important contributor to symptoms and disability. The evaluation of axonal degeneration is still a major challenge in MS, and with the advent of newer and more aggressive disease-modifying therapies (DMTs) that can delay disability, there is a real need for a reliable test to measure disease status. Most of the algorithms guiding disease monitoring and treatment switching today are based on the occurrence of clinical relapses, disability progression, MRI activity, and more recently brain atrophy, however, many of these parameters are retrospective, imprecise, and often fail to predict individual disease progression and therapy response. Therefore, a new biomarker that can reflect tissue damage and allow monitoring of subclinical disease activity is highly desirable.

A notable example of a fluid biomarker that is already in clinical use in MS is the oligoclonal bands. Yet, no fluid biomarker has established clinical use in routine disease monitoring and prediction in MS. Because of this unmet need, many studies have been performed towards this purpose, among these are the studies that evaluate neurofilament’s role in MS, which has shown some promising results. Neurofilaments are neuronal-specific heteropolymers that are particularly abundant in axons. Their functions include the provision of structural support and maintaining the size, shape, and caliber of the axons. Neurofilaments consist of a triplet of light (NfL), medium (NfM), and heavy (NfH) chains.
(~145 kDa), and heavy (~200 kDa) refer to the molecular weight of the filaments.8

Disruption to the axonal membrane releases Nf into the interstitial fluid and eventually into the cerebrospinal fluid (CSF) and, subsequently blood.9,10 Hence, measuring intracellular cytoskeletal proteins like NfL and NfH seems to be a comprehensive way to assess the extent of axonal damage within the central nervous system. In humans, neurofilaments were first used as indicators of neuronal damage in amyotrophic lateral sclerosis (ALS) and Alzheimer’s disease.11 Subsequently, neurofilaments levels were found to be higher in CSF of MS patients compared to controls, suggesting these proteins could also be a potential biomarker for MS disease activity.12,13,14 Recent studies have focused on sampling Nf in serum instead of the relatively invasive CSF, and fortunately, a lot of them have shown promising results for associating serum Nf levels with outcomes related to MS disease activity, progression, treatment response, and prognosis.15,16,17,18

Our study was done to establish the value of serum Nf as a biomarker in MS, we aim to examine the effects of disease duration, activity (clinical and radiological), disability, and treatment on serum NfL and NfH levels, using available resources in our region.

METHODS

A case-control study was conducted in the Middle-Euphrates-Neuroscience Centre, Al-Najaf, over a period of 4 months from June to October 2019.

Inclusion criteria

Fifty six cases aged (16-45 years) with RRMS (no.=49), SPMS (no.=6), and PPMS (no.=1) were included in the study. All patients fulfilled the revised McDonald’s criteria (2017) for the diagnosis of MS.19

Forty-four age and gender-matched, healthy volunteers with no previous chronic disease were included as controls. All the participants in the study voluntarily provided verbal informed consent.

Exclusion criteria

The exclusion criteria were: 1. Patients with neurologic (other than MS), inflammatory, and psychiatric conditions; 2. Patients with co-morbid cardiovascular risk factors such as DM and hypertension20, 3. In Patients with pseudorelapse19 a pseudorelapse is defined as the recurrence of symptoms from a previous clinical relapse or a subclinical lesion; 4. Extremes of age; as age can physiologically increase Nf levels by 2.2% per year, and above the age of 60 sNf levels increase greatly.21

Clinical assessment

History and clinical examinations were performed for all participants in the MS clinic unit. Age, gender, type of MS, duration since the onset of symptoms, disease severity, disability, and type of treatment were recorded for each case. Clinical neurological assessment was done using the revised McDonald’s criteria (2017) for diagnosis of cases19 and EDSS22 for assessment of disability. MS disease severity was calculated by using the EDSS score and disease duration for each patient applied in MSSS.23 Patients were also assessed for clinical relapse in the last 3 months.24

Blood sampling

For MS cases and controls, venous blood samples were drawn into an additive-free plastic blood collection tube and allowed to clot for a minimum of 30 minutes and a maximum of 120 minutes at room temperature. The serum was removed after centrifuging, aliquoted, and stored at -20℃. Neurofilament light and heavy chains were measured by sandwich ELISA method (Human neurofilament light polypeptide and Human phosphorylated neurofilament heavy polypeptide ELISA kit, Elabscience Biotechnology Inc.) in a board-certified lab in Al-Najaf city.

Radiological assessment

A standard MRI protocol for MS with IV gadolinium (Gd)25 (using 1.5 tesla MRI scanner, Siemens Medical Systems, Germany) was done within 3 weeks26 of the venous blood sampling for each patient and was analyzed for the presence and number of gadolinium lesions by an experienced radiologist.

Statistical analysis

The normality of the distribution of continuous variables was tested by the one-sample Kolmogorov-Smirnov test. Means and standard deviations (SDs) were given for the normally distributed variables, while median, minimum, and maximum values were given for variables that were not normally distributed. Frequencies and percentages were used to present categorical
The differences between NfL and NfH levels in cases and controls were assessed using independent samples t-test assuming unequal variances. The Mann Whitney U test was used to test the NfL and NfH levels in patients with positive and negative MRI-enhanced gadolinium lesions as well as to compare those levels for patients in remission to the levels of patients with clinical relapse. The Spearman rho test was used to test whether different variables correlate with NfL and NfH levels because the samples weren’t normally distributed. The two-tailed p-value was 0.05 for all the tests used. All the tests were made using SPSS 26.0, IBM, NY, US.

RESULTS

**Demographical data of study and control group**

Fifty-six cases were enrolled in the study, the mean age was 34.3 (SD = 9.1) years with a median disease duration of 3 (0 – 14) years. Forty-four subjects were included as controls with a mean age of 33.7 (SD = 7.2). The male:female ratio was 1:3 for the cases and 1:2 for controls. Other patient characteristics are demonstrated in Tables 1 and 2.

Eighteen patients (32.1%) were on treatment with interferon beta-1a, and the same number of patients were on interferon beta-1b. The rest of the patients were on natalizumab (5 patients, 8.9%), fingolimod (3 patients, 5.4%), or no treatment (12 patients, 21.4%).

Most MS cases (n=48; 85.7%) had no enhancing gadolinium lesions on MRI as demonstrated in Table 2. Forty-four patients (74.6%) were in remission at the time of data collection, and twelve patients (25.4%) were in clinical relapse.

**Results of serum NfL and NfH**

The mean NfL level for cases was 133.3 (SD = 62.6) pg/ml which is significantly higher than that for controls (Mean = 80 pg/ml; SD = 22.5; p-value <0.001). The mean NfH level was 3654.5 (SD = 567.8) pg/ml for cases which is also significantly higher than the levels obtained from healthy subjects (408.8 pg/ml; SD = 343.5; p-value < 0.001) as shown in Table 3.

Table 1: Demographic characteristics of MS patients and healthy controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases N=56</th>
<th>Control N=44</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Mean age in years (SD)</td>
<td>34.3 (9.1)</td>
<td>33.7 (7.2)</td>
<td>0.81</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Male N (%)</td>
<td>14 (23.7)</td>
<td>14 (29.8)</td>
<td>0.45</td>
</tr>
<tr>
<td>Female N (%)</td>
<td>42 (71.2)</td>
<td>30 (63.8)</td>
<td></td>
</tr>
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</table>

Table 2: Disease characteristics among MS patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean EDSS (SD)</td>
<td>1.77 (1.8)</td>
</tr>
<tr>
<td>Mean MSSS (SD)</td>
<td>3.2 (2.8)</td>
</tr>
<tr>
<td>MS Type:</td>
<td></td>
</tr>
<tr>
<td>RR MS, N (%)</td>
<td>49 (87.5)</td>
</tr>
<tr>
<td>SP MS, N (%)</td>
<td>6 (10.7)</td>
</tr>
<tr>
<td>PP MS, N (%)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Number of gadolinium lesions, N (%)</td>
<td></td>
</tr>
<tr>
<td>No lesion</td>
<td>48 (85.7%)</td>
</tr>
<tr>
<td>1</td>
<td>4 (7.1%)</td>
</tr>
<tr>
<td>2</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>3</td>
<td>2 (3.6%)</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>1 (1.8%)</td>
</tr>
</tbody>
</table>

EDSS = expanded disability status scale; n = number; MS = multiple sclerosis; MSSS = multiple sclerosis severity score; RR = relapsing-remitting; SP = secondary progressive; PP = primary progressive; SD = standard deviation.
Serum Nf correlation to image findings

The mean NfL and NfH levels were not significantly different between patients with negative and positive gd-enhanced MRI lesions, as shown in Table 4.

Serum Nf correlation to MS severity and other demographic data

Correlation analysis, using Spearman rho statistic, did not demonstrate significant correlations between NfL levels and EDSS, MSSS, age, or duration of the disease. The same goes for NfH levels, as shown in Table 5.

There was no significant association between clinical disease activity and NfH (p = 0.75) or NfL (p = 0.71) levels as demonstrated in Figure 1.

Serum Nf correlation to MS treatment status

There was no significant difference between NfL and NfH levels and different treatment groups (Interferon beta-1-a, interferon beta-1-b, natalizumab, fingolimod and no treatment) in MS patients (NfL: P value = 0.8; NfH: P-value = 0.73). Boxplots of NfL and NfH in different treatment groups are shown in Figure 2.

DISCUSSION

This study evaluates serum NfL level as a biomarker in MS by comparing these levels in a sample of Iraqi MS patients to that of age and gender-matched controls using commercially available ELISA. Moreover, it compares serum NfL to NfH levels and investigates the relationship between these 2 assays and disability, brain MRI activity, clinical disease activity, severity, and treatment status. Importantly, our method allows us to make use of the readily available patient blood samples, instead of the relatively difficult CSF sampling.

The male-to-female ratio in the study sample was 1:3 which is reflective of the female predominance seen in MS. The duration of the disease in this study did not correlate with serum NfL and NfH levels. Meta-analyses of case-control studies reported limited data in subgroup analysis to reach a consensus regarding whether NfL and NfH levels were correlated with disease duration.

As for MS type, there is heterogeneity in reports comparing Nf levels in PMS with that of RRMS cases, however, some studies have reported NfL to be higher or increase more rapidly in PMS compared to RRMS, whilst others have found no such correlation. In this study, NF levels were not different between patients with RRMS and PMS, and this could be attributed, at least in part, to the fact that most patients in this study were in the RRMS subgroup while only seven patients collectively had the progressive subtype.

We have found that serum NfL and NfH levels were significantly higher in MS cases than in controls. These findings are consistent with a recent meta-analysis by Cai et al. and other studies.
Siller et al. found that serum NfL levels correlated significantly with T2 lesion volume and with the presence and number of Gd-enhancing lesions; Kuhle and colleagues also have reached a similar conclusion. Unfortunately, the software that analyzes the volume of T2 lesions and brain atrophy is not available in our center so we could not assess that correlation, however, we compared serum NfL & NfH levels for different gd-enhanced MRI results, and in this regard, we could not

Table 5: Correlations between NfL and NfH levels and other variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NfL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDSS</td>
<td>-0.06</td>
<td>0.7</td>
</tr>
<tr>
<td>MSSS</td>
<td>-0.07</td>
<td>0.59</td>
</tr>
<tr>
<td>Age</td>
<td>-0.11</td>
<td>0.4</td>
</tr>
<tr>
<td>Duration</td>
<td>-0.19</td>
<td>0.14</td>
</tr>
<tr>
<td>NfH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDSS</td>
<td>-0.06</td>
<td>0.63</td>
</tr>
<tr>
<td>MSSS</td>
<td>-0.13</td>
<td>0.32</td>
</tr>
<tr>
<td>Age</td>
<td>0.1</td>
<td>0.46</td>
</tr>
<tr>
<td>Duration</td>
<td>-0.1</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Figure 1 A: Mean NFL-H in pg/ml among MS patients with clinical relapse in comparison to patients in remission.

Figure 1 B: Mean NFL-L in pg/ml among MS patients with clinical relapse in comparison to patients in remission.
detect a significant association between serum Nf levels and MRI findings; however, the presence of significantly higher NfL and NfH levels in patients without Gd-enhanced lesions on MRI compared to those in healthy controls, suggests that Nf levels may reflect ongoing neuronal damage and loss, independent of detectable inflammatory activity.35 Damasceno et al. and Gresle et al. have reached a similar conclusion in their analysis.36,33

Existing data on the relation between Nf and clinical outcomes in MS are limited to relatively short-term correlations. Disanto et al. showed that serum NfL was independently associated with EDSS assessments.17 Other studies also showed that serum and CSF NfL levels were correlated with disability scores over time.36,34 More recently, using large nationwide population-based data, Manouchehrinia et al. found that elevated NfL levels at the time of MS diagnosis are associated with the risk of long-term sustained disability development.37 However, we were unable to reach a similar finding in our study; serum NfL and NfH could not be proved to correlate with EDSS or MSS scores. However, our results go in line with a study by Arrambide and colleagues which demonstrated that serum NfL levels were not correlated with disability in MS.38 As for clinical disease activity, our study did not have the power to identify a significant difference in Nf levels between patients in remission and those with clinical relapse. On the other hand, many recent studies have consistently reported higher serum NfL levels in MS cases with disease activity compared to MS cases in remission. However, Cantó et al. reported no such association between serum NfL and disease activity in MS.16

There could be a few explanations for these inconsistencies: First, the sampling in our study was done at a single point in time and some reports suggest that Nf may be released periodically rather than continuously, so, cross-sectional sampling could underestimate the proportion of patients with intermittently high Nf serum titers; Secondly, the relatively small sample size and the
fact that many patients were clinically stable or on treatment, could be implicated in these results; And finally, the analysis of neurofilament levels in our study was undertaken by commercially available ELISA kits unlike most of the recent studies that have used SIMOA method for serum sample assay; which is more sensitive than ELISA\textsuperscript{39}, unfortunately, SIMOA is not available in our region.

Regarding treatment effects, longitudinal NFL reductions have been reported for most established treatments for relapsing and progressive MS, these include dimethyl fumarate\textsuperscript{40}, fingolimod\textsuperscript{41}, natalizumab\textsuperscript{42} siponimod\textsuperscript{43}, ocrelizumab\textsuperscript{44}, and ofatumumab\textsuperscript{45} among others. However, in this analysis, NFL and NFH levels were not significantly different between newly diagnosed patients and those already on treatment or between various treatment groups. This controversy in results could be justified by the fact that the participants in this study have been sampled only once, while a considerable treatment effect on Nf would be rather apparent if a frequent sampling method had been adopted instead.

In conclusion, in this study, we could not find a significant correlation between serum NFL levels and disease parameters in MS including disability, and clinical and radiological disease activity; however, serum NFL levels were significantly higher in MS cases compared to healthy controls.

**DISCLOSURE**

Ethics: This study was approved by the Institutional Review Board at Kufa University. Written informed consent from the patients was obtained.

Financial support: None

Conflict of interest: None

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