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Original Article

Assessment of Serum Neurofilament Light Chain Protein in Multiple Sclerosis Patients

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ABSTRACT

Background: .Serum neurofilament light chain (NFL) has been the subject of intensive research as a potential biomarker of treatment response as well as prognosis among multiple sclerosis cases because it can reflect disease activity in the clinical follow up of these patients and measured in serum.
Aim: to assess serum NFL in MS cases and its relation with disease severity & magnetic resonance imaging (MRI) finding in these patients.
Subjects and methods: Fifty MS patients were included in a case-control research conducted at three hospitals in Egypt: Zagazig University Hospital, ALMabara Hospital for Health Insurance in Zagazig, and Mansoura New General Hospital. Diagnosis of MS was done according to Modified McDonald criteria. Patients were divided into 25 patients with relapsing–remitting multiple sclerosis (RRMS), 18 patients with secondary progressive multiple sclerosis (SPMS), 7 patients with primary progressive multiple sclerosis (PPMS) & 25 sex and age matched healthy controls.
Results: There is a statistical significance increase serum NFL among cases than control group and a significance increase of Serum NFL among RRMS than SPMS and PPMS groups.
Conclusion: The results showed that NFL levels were associated with disease severity as assessed by Expanded Disability Status Scale (EDSS) as well as MRI findings in MS patients.
Keywords: Neurofilament, Light Chain Protein, Multiple Sclerosis.

INTRODUCTION

Neuroaxonal damage in multiple sclerosis is highly correlated with clinical and MRI events, as well as prognosis. The NLC of neurofilaments Structure in the brain and spinal cord is supported by NFL, a protein found in abundance in the neuronal and axonal cytoskeleton. Increased NFL has been found in a variety of neurodegenerative, traumatic, and ischemic brain illnesses in addition to inflammatory conditions [1].

Increased levels of NFL were found when comparing patients with more than 30 different neurological diseases to healthy controls. Therefore, elevated NFL levels are indicative of axonal injury in general, regardless of the cause. On the other hand, the absolute values and/or either or temporal dynamics can suggest various potential interpretations. When serum NFL levels are elevated beyond what would be expected in otherwise stable MS patients, it is important to rule out other causes and comorbidities including head trauma, polyneuropathy, or microvascular CNS lesions. Earlier research from a smaller cohort did not find any association between NFL levels and neuropsychiatric or cognitive problems [2].

After an axonal injury, NFL proteins are secreted into the CSF and, to a lesser extent, the peripheral circulation (around 2 percent). Researchers have been looking at neurofilament proteins for almost 20 years, but at first, the results could only be applied to animal models because they relied on obtaining CSF samples. Since 2017, researchers studying MS have made increased use of cutting-edge analytic techniques (such as single molecule array, SIMOA) that allow them to detect quantities in the low picogram/milliliter range in patient blood and plasma [3].

Thus we aimed at this work to assess serum NFL in MS cases and its relation with disease severity & magnetic resonance imaging (MRI) finding in these patients.

SUBJECTS AND METHODS

Fifty patients with multiple sclerosis, diagnosed according to the Modified McDonald criteria, were included in this case control study. The study was conducted in the Neurology Department, Zagazig University Hospitals, AL Mabara Hospital for Health Insurance in Zagazig, and Mansoura New General Hospital.

Included subjects were subdivided to 25 patients as RRMS, 18 patients as SPMS, 7 patients as PPMS using the Modified McDonald criteria and 25 Age and sex matched healthy individuals.

All of the chosen participants were given thorough explanations of the study's goal and anticipated advantages. The Declaration of Helsinki, the World Medical Association's code of ethics for studies involving humans, guided the conduct of this work. Participants provided verbal consent after being fully informed, and information confidentiality was guaranteed. IRB approval No (6407).

Methods:

All cases were subjected to:

Complete history taking including (duration of illness, duration and course of disease, numbers of attacks), complete general examination, complete neurological examination, and Kurtzke's Expanded Disability Status Score (EDSS). This scale has eight Functional Systems (FS) that neurologists can use to quantify a patient's level of functioning impairment [4].

Investigations:

1) Laboratory investigation:

(a) Routine lab. (Complete blood count, Liver function, kidney function & blood glucose)

(b)Serum neurofilament light chain protein concentration measured by ELISA [5].

2) Radiological investigation: MRI scan of brain 1.5 Tesla (with contrast).

Statistical analysis

The data was analyzed using SPSS for Windows (Statistical Package for the Social Sciences) (Standard version 26). A one-sample Kolmogorov-Smirnov test was performed to check for data normality. Both quantitative and qualitative data were displayed similarly. Chi-square tests were used to look for patterns of association between categorical variables. For properly distributed data, the continuous variables were displayed as mean SD (standard deviation), and for non-parametric data, the median (range) was used. Both the parametric Student t test and the non-parametric Mann Whitney test were used to compare the two groups, while the Kruskal-Wallis test was employed to analyse the data from more than two groups (non-parametric). The continuous data were correlated using the Spearman method. The ROC curve was used to evaluate the sensitivity and specificity at varying thresholds. The level of significance at which all of the aforementioned statistical tests are considered to be significant is 5%. When p was less than 0.05, the outcome was considered to be significant. The more significant the results, the smaller the p-value [6].

RESULTS

There is a statistically significant increase of age among SPMS than RRMS and PPMS and control groups. Mean duration of illness increased significantly among SPMS cases compared to RRMS and PPMS cases. Regarding date of last attack (years); there is a statistically significant increase in mean date since last attack among SPMS cases compared to other types. Median number of relapses illustrates statistically significant higher median number among SPMS group than RRMS & PPMS groups. Regarding EDSS; there is a statistically significant increase in median EDSS among SPMS cases compared to RRMS and PPMS cases (table1).

The use of different drugs is significantly different amongst the groups examined ($p<0.001$). Among SPMS group ;94.4% use Rituximab and 5.6% Cyclophosphamide and among PPMS group ; 100% Ocrelizumab and among RRMS group ; 52% use Fingolimod , 16% use Interferon beta-1a, 12% use Teriflunamide , 8% use Interferon beta-1b & Ocrelizumab and 4% use Interferon beta-1a (table2).

There is a statistically significant increase of serum NFL among cases than control group ($p<0.001$) (table3, fig1).There is a statistically significant increase of Serum NFL among RRMS than SPMS and PPMS groups (68.9, 28.94 &20.68, respectively) (table4).There was statistically significant higher median serum NFL among RRMS than SPMS and PPMS than control group

There is statistically significant higher median number of patches in Periventricular, Juxta-cortical and cortical lesions among SPMS group followed by RRMS and PPMS .GD enhanced lesions and newly emerging lesion are a statistically significantly higher among RRMS than SPMS group (table4).

Table (1): comparison of age between MS subtypes

	Cases MS group n=50	control group n=25	RRMS group n=25	SPMS group n=18	PPMS group n=7	F	Overall p	Post Hoc Tukey test
Age/years mean±SD (Range)	32.84±8.79 20-49	34.92±10.96 18-55	27.80±5.35 21-42	42.33±4.68 33-49	26.42±5.56 20-38	48.01	0.888 <0.001*	p1= 0.377 p2=0.002*(S p3=0.002*(S p4=0.01*(S)

Table (2): comparison of current medications used in different MS subtypes

current medications	RRMS group n=25	SPMS group n=18	PPMS group n=7	MC	Overall p	post Hoc
Rituximab	0	17(94.4)	0	85.78	P<0.001*(S)	p1<0.001*(S) p2=0.001*(S) p3=0.001*(S)
Interferon beta-1a	4(16)	0	0			
Ocrelizumab	2(8.0)	0	7(100)			
Fingolimod	13(52)	0	0			
Cyclophosphamide	0	1(5.6)	0			
Interferon beta-1b	2(8)	0	0			
Interferon beta-1a	1(4.0)	0	0			
Teriflunomide	3(12.0)	0	0			

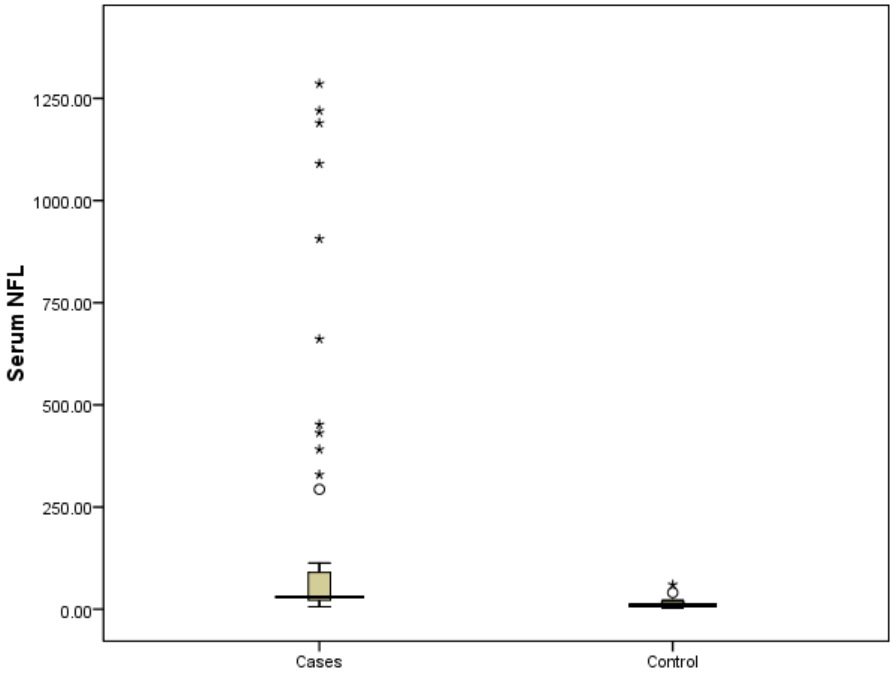
Table (3): Comparison of Serum NFL between studied groups.

	Cases (MS) group n=50	Control group n=25	test of significance
Serum NFL median (min-max)	29.72(5.82-1286)	9.7(2.8-60)	Z=5.45 P<0.001*

Table (4): comparison of Serum NFL between MS phenotypes & control group.

	RRMS group n=25	SPMS group n=18	PPMS group n=7	KW	Overall p	Post Hoc
Serum NFL median (min-max)	68.9 (5.82-1286)	28.94 (14.5-113.1)	20.68 (19.5-23.15)	10.31	0.006*	p1<0.001(S) p2=0.02*(S) p3<0.001*(S)

Figure (1): comparison of Serum NFL between studied groups



DISCUSSION

Neurofilament Light Chain is a subunit of neurofilaments that constitute the neuronal and axonal cytoskeleton in the central nervous system (CNS) and the peripheral nervous system (PNS) and is released into the cerebrospinal fluid (CSF) and blood when neuronal and axonal damage occurs [7].

When we compared the age distributions of the groups, we found no statistically significant differences. This matches with previous study which illustrated that MS group and control group were in the same age range from (20 to 55 years old) as the average onset age for MS (mean age of onset, 20-30 years) [7].

When we compare age between MS phenotypes we found that there was a statistically significant increase of age among SPMS than RRMS as SPMS typically develops 10–15 years after RRMS onset like what was published by Dobson and Giovannoni [7] in their previous study.

Regarding types of MS among the patients in our study, we found that the most frequent MS type was RRMS (50%) followed by SPMS (36%) while PPMS was found only in 14.0% of the cases .This matches with the findings of Engelhard et al [8] who found that 73% of patients were diagnosed with RRMS, 8% with PPMS, and 19% with SPMS.

As regard the disease duration, our study showed that there was increase in disease duration among SPMS patients than RRMS and PPMS. This is similar to other studies that stated that within 15 years of being diagnosed with RRMS, 50% of people will acquire secondary progressive multiple sclerosis (SPMS), and up to 65% of people will get SPMS after 30 years.

Multiple sclerosis (MS) typically follows a relapsing-remitting course, with gradual disability worsening over time that is not associated with clinically apparent relapses, and is therefore referred to as secondary-progressive MS (SPMS). This transition can occur in as many as 80% of patients within 20 years. [9, 12].Regarding EDSS, our study results showed a statistically significant increase in median EDSS among SPMS cases compared to RRMS and PPMS cases. This matches with a study done by Leporeet al. [10] who showed that most RRMS patients presented with lower EDSS.

Our results demonstrated that there is a statistically significant higher median number of MRI plaques in Periventricular, Juxta-cortical and cortical lesions among SPMS group followed by RRMS and PPMS. Gadolinium enhanced lesions and newly emerging lesion are also statically higher among RRMS than SPMS group, as Gadolinium-enhancing and T2 lesions reflect disease activity which is prominent features of RRMS subtype [11].

The serum levels of NFL were significantly higher in the patients compared to the controls in our study. This correlates with the study of Ning andWang [12] who found that serum NFL levels

are significantly higher in MS patients in comparison to age-matched controls.

Bridel. et al.[13] reported that in a group of 90 MS patients, NFL was assessed twice at regular visits untreated relapsing remitting MS (uRRMS), secondary progressive MS, treated relapsing remitting MS (tRRMS), SPMS, primary progressive MS ,as well as age-matched healthy control, the mean NFL was higher in all MS subtypes than healthy control.

Another study reinforced our results that patients with MS had higher levels of serum NFL when compared to healthy controls, even before the onset of clinical symptoms by several years [14].Our current study results also showed a statistically significant increase of Serum NFL among RRMS than SPMS and PPMS groups (68.9 , 28.94 and 20.68 , respectively).

The same was also reported by van den Bosch.et al. [15] who reported that, the presence of acute axonal damage in lesions was related to higher NFL levels and the amount of acute axonal damage was higher in active lesions.

Martin. et al. [16]mentioned that When comparing RRMS and PPMS, mean NFL levels were considerably greater in relapsing disease, and in relapse they were twice as high as they were during remission.

Evidence suggests that NFL levels are positively correlated with relapses and its level decline in remission [17].

Conclusion:

NFL levels are significantly increased in MS cases in comparison to healthy control.

There is a statistical significance increase of Serum NFL among RRMS than SPMS and PPMS groups.

Conflict of interest:

No potential conflict of interest was reported by the authors.

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