Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://dx.doi.org/10.21608/zumj.2019.15900.1424 Volume 28, Issue 6, November 2022(45-51) Supplement Issue
Manuscript ID ZUMJ-1908-1424 (R6)
DOI **10.21608/zumj.2019.15900.1424**

ORIGINAL ARTICLE

SERUM PHOSPHORYLATED NEUROFILAMENT-HEAVY CHAIN LEVELS IN MULTIPLE SCLEROSIS PATIENTS

Adel Saed Abd Elghafar [1], Mahmoud El Sayed El Ebeary [2], Magdy Abd Elhamed Aidaros [3], Nagwa Mohammed shawky[4], Doaa abd Elsattar Mohammed [5]

[1] Professor of Neurology Faculty of medicine, Zagazig University, Egypt.
[2] Professor of Neurology Faculty of medicine, Zagazig University, Egypt.
[3] Professor of Neurology Faculty of medicine, Zagazig University, Egypt.
[4] Professor of Clinical pathology Faculty of medicine, Zagazig University, Egypt.
[5] Assistant Lecture of Neurology Faculty of Medicine, Portsaid University, Egypt.

Corresponding author:
Doaa Abd Elsattar Mohammed
E.mail :
doaa_sattar2010@yahoo.com

Submit Date 2019-08-22
Revise Date 2019-12-28
Accept Date 2019-12-31

ABSTRACT

Background: Axonal injury is the main pathological correlate of progressive neurological disability in multiple sclerosis (MS). Serum phosphorylated neurofilament-heavy (pNF-H) might be useful marker to monitor axonal loss in MS, but we need to correlate its serum level with different disease parameters such as disease course, disa bility and treatment effect.

Aim: To asses whether serum pNF-H levels are associated with clinical and paraclinical measures of disease severity in order to validate this protein as a likely surrogate marker for axonal injury in MS patients.

Subjects and methods: Study included 40 patients and 40 healthy control. All Subjects in this study were subjected to full history taking, general examination including Body mass index (BMI), neurological examination and scoring according to the Expanded Disability Status Score (EDSS), Routine Laboratory Investigations, measuring pNF-H levels and neuroradiological assessment using Magnetic resonance image (MRI) to confirm the diagnosis of MS**.**

Results: The serum levels of pNF-H peptide were higher in MS patients compared to healthy controls. The higher levels are in the progressive subgroup and these levels were significantly correlated with EDSS, BMI, disease duration and age of the patients.

Conclusion: The increase in pNF-H titer in MS patients and in the progressive subgroup together with the correlation of pNF-H levels with all clinical parameters suggests that cumulative axonal loss was responsible for sustained disability and that high PNF-H level may be used as an indicator of poor prognostic value.

Keywords: multiple sclerosis, axonal damage, neurofilament – H.

INTRODUCTION

Multiple Sclerosis (MS) is the commonest non-traumatic disabling neurological disease affecting young adults. The incidence of MS and the socioeconomic impact of the disease are increasing worldwide. The cause of MS and mechanisms behind this increase remain unclear, although complex gene- environment interaction may play a significant role [1].

The clinical manifestations can include visual affection, exhaustion, depression, hemiplegia,paraplegia, parathesia and cognitive impairment. Early symptoms began in young adulthood, a time when diagnosis of an unpredictable, chronic neurological disease with significant and progressive disability is almostly devastating and unexpected [2].

Moreover, axonal injury is recognized as the main pathological cause of progressive neurological deterioration in MS [3].

At present markers for axonal injury are not used as routine investigations for monitoring disease activity in MS patients. Magnetic resonance imaging utilizing T2-weigheted imaging and Gadolinium enhanced T1-weighted imaging are

the most commonly used diagnostic and monitoring tool for MS [4].

Several studies are focusing now on the detection of neuronal/axonal proteins in cerebrospinal fluid (CSF) or blood as biomarkers of axonal injury. They are based on the concept that degenerating axons release their contents into the surrounding extracellular space and that some of these axonal components might be abundant and stable enough to be detected with appropriate assays. The detection of these components would provide useful means to assess the occurrence and degree of axonal degeneration in MS and this information could be useful for predicting and assessing the disease progression and for evaluating the efficacy of therapeutic strategies aimed for preventing axonal injury and loss [5, 6].

Neurofilaments (NFs) are the major building proteins of neurons. There are three major neurofilament protein subunits, (light NF-L medium NF-M and heavy NF-H) that form the cytoskeleton of the axon. light NF-L and NF-H are released from damaged axons and easily detected in CSF or serum by the enzyme- linked immunosorbent assay (ELISA) methodology and the higher levels of both proteins are associated with worst clinical outcomes [7].

SUBJECTS AND METHODS

This case–control comparative study was conducted on 80 subjects; The study was approved by Institution Review Board at Zagazig University. Patients were recruited from internal section and outpatient clinic of Zagazig University Hospitals between the period February 2016 to february 2018. fourty healthy individuals were participated as a control group, selected from general population through personal communication. Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University.

(Patients group)(group 1): 40 patients with clinically definite Multiple Sclerosis. Data about age, gender, age of onest, duration, course of the disease, type of disability and the pharmacological treatment, divided into, subgroup 1a: Included 10 patients with first demyelinating event (FDE). subgroup 1b: Included 16 patients with relapsing remitting multiple sclerosis (RRMS).subgroup 1c: Included 9 patients Secondary progressive multiple sclerosis (SPMS).subgroup 1d: Included 5 patients primary progressive multiple sclerosis (PPMS). Controls group (Goup 2): Forty healthy peoples with age and sex matching ,should be neurologically normal during physical examination.

Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria:

Definite MS according to the 2010 revised McDonald diagnostic criteria for MS [8].

Exclusion criteria : Patients having any other medical diseases (neurological, immunological, cardiac, hepatic, and renal), previous head trauma, pregnancy or transform patients (patients shifted from interferon to fingilimod) were excluded from our study.

Patients were subjected to the following:

Complete history taking, full general including BMI, neurological examination according to the sheet in our department and evaluated with Expanded Disability Status Scale for measuring disease sevirity.

Laboratory investigations*:* Both routine and special laboratory investigations were done at Clinical Pathology Department, Zagazig University Hospitals.

Routine Laboratory Invesigations : complete blood picture, Liver and kidney function tests, Erthrocyte sedimentation rate, Fasting and postprandial blood sugar and lipid profile.

Special laboratory investigations : Serum phosporylated neurofilament-H chain (pNF-H) levels of both patients and control groups were analyzed using The enzyme-linked immunosorbent assay (ELISA) kit (EnCor Biotechnology Inc, Gainesville, Florida, USA), according to the manufacturer instructions [9,10].

Statistical analysis

Data were analyzed using Statistical Program for Social Science, version 24 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean±SD. Qualitative data were expressed as frequency and percentage. The following tests were done,Mann-Whitney U test (MWT) Pearson's correlation coefficient (r) test was used for correlating data: Probability (P value) (a) P value less than 0.05 was considered significant. (b) P value less than 0.001 was considered as highly significant.(c) P value greater than 0.05 was considered insignificant [11].

RESULTS

This case control comparative study included forty MS patients , their age ranged between 19 to 61 years with M ± SD = 38.7 ± 12.5 ,37.5% (15 patients) were males, 62.5% (25 patients) were females (table 1).

The age of onset of the disease in patients group ranged between 17 to 61 years old with M ± SD = 30.70 ± 9.51.Our patients manifested by various clinical presentation the most common presentation was motor disturbance (57.5%), followed by sensory manifestation (17.5%), followed by vestibulocerebellar symptoms (12.5%), followed by visual disturbance (7.5%) and lastly diplopia (5%). The disease duration ranged from 0 to 25 years with M ± SD = 7.76 ± 7.03 . The body mass index of patients M ± SD = 24.21 ± 2.66. Among these patients, only one patient had positive family history (2.5 %). Evaluation of disese severity according to EDSS, thirty three patients (82.5 %) step 1.0 to 4.5 whom are fully ambulatory, seven patients (17.5%) step 5.0 to 9.5 whom had impairment to ambulation. As regards treatment twenty four patients (60%) under treatment (ten patients 41.6% are on fingilomod, fourteen on interferon 58.4% the other sixteen patients did not receive specific treatment for MS apart from corticosteroid during the attacks (table 1).

There was statistical significance difference between patients of different MS subtypes (FDE, PP, RR and SP) regarding BMI and EDSS and highly statistically significant difference regarding to age and disease duration but there was no significant difference between them regarding to sex (figure 1). There is highly statistically significant difference in the serum pNF-H levels between MS patients and controls (pNF-H levels in the patients is significant higher than control group) (table 2).

There was highly Statistically significant difference in the serum levels of pNF-H between MS subtypes (Figure 2). There was direct correlation between serum pNF-H levels and age of patients, BMI , disease duration ,EDSS and age of controls(table 3). There was significant difference in serum pNF-H between treated and non-treated patients with the lowest levels in patients treated with fingolimod (figure 3).

Table (1): Demographic data of our patients

Clinical finding		**MS Group N=40**
Gender	Male	15 (37.5%)
	Female	25 (62.5%)
Age		38.7 ± 12.5

MS = Multiple sclerosis

Table (2): Clinical characteristic of the patients (N=40).

Clinical data	**SD or %±M**
Age of onset range in years (M ± SD)	17 – 61 (30.70 ± 9.51)
Clinical presentation	No
Motor disturbance	23
Sensory manifestation	7
Vestibulocerebellar symptoms	5
Visual disturbance	3
Diplopia	2
Disease duration range in years (M ± SD)	0 – 25 (7.76 ± 7.03)
Body mass index	24.21 ± 2.66
Family history	
Positive	1 (2.5 %)
Negative	39 (97.5)
EDSS	
EDSS steps 1.0 to 4.5	33(82.5%)
EDSS steps 5.0 to 9.5	7(17.5%)
Treatment	
Treated	24 (60%)
Untreated (not on modifying treatment)	16 (40%)
Treatment type	
Fingilomod	10 (41.6%)
Interferon	14(58.4%)

EDSS= Expanded disability Status Scale MRI= Magnetic Resonance Imaging

Table (3): Serum phosphorylated -heavy neurofilament levels in patients group and controls group

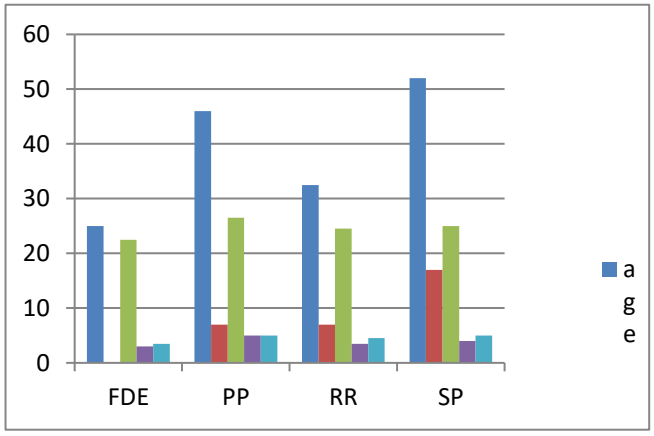
Group	MS group			Control group			Total			MWT	P
	N	Median	Range	N	Median	Range	N	Median	Range		
pNF-H	40	0.887	0.401-12.285	40	0.575	0.261-0.669	80	0.629	0.261-12.285	6.9	**<0.001**

MS= multiple sclerosis MWT= Mann-Whitney U test pNF-H= phosphorylated neurofilament heavy chain

Table (4) Correlations between serum phosphorylated - neurofilament heavy chain level and other studied parameters

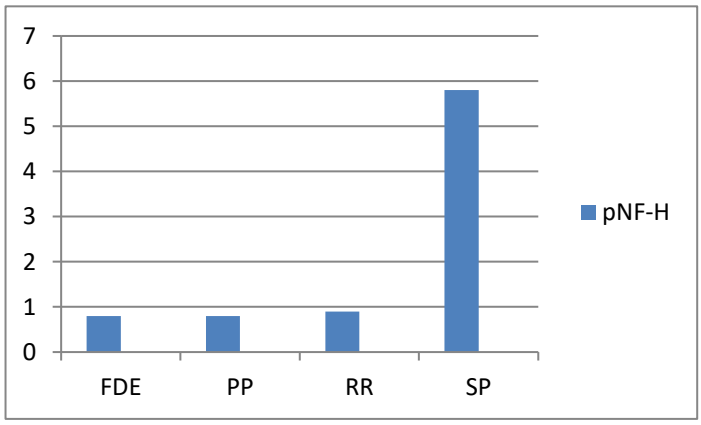
MS	pNF-H	
	r	P
Age of patients	0.531	**<0.001**
BMI	0.479	0.002
Diseae duration	0.657	**<0.001**
EDSS	0.531	**<0.001**
Age of controls	0.4045	**0.009**

r = Correlation Coefficient EDSS= expanded disability status scale
BMI= body mass index pNF-H= phosphorylated - neurofilament heavy chain



FDE= first demyelinating event PP= primary progressive MS
SP = secondary progressive MS RR= relapsing remitting MS
EDSS= Expanded disability status scale BMI=body mass index
DD= disease duration

Figure (1): A comparison between the clinical and demographic finding in different Multiple sclerosis subtypes



FDE= first demyelinating event PP= primary progressive MS
SP = secondary progressive MS RR= relapsing remitting MS
pNF-H= phosphorylated neurofilament heavy chain

Figure (2): Comparison of serum phosphorylated - neurofilament heavy chain levels according to multiple sclerosis subtype.

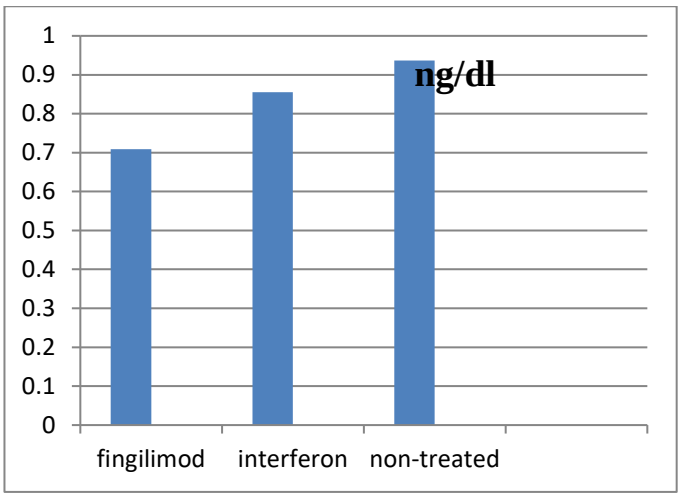


Figure (3): Comparison of serum phosphorylated - neurofilament heavy chain levels in multiple sclerosis group as regards treatment (fingilimod or interfreron)

DISCUSSION

Phosphorylated neurofilament heavy chain is an ideal biomarker of axonal loss because **1)** it is resistant to proteases degradation before or following its release, **2)** it is expressed specifically in axons **3)** it is abundant enough to be readily detectable after the significant dilution that occurs when it released into fluid compartments such as cerebrospinal fluid (CSF) or serum. After axonal injury, pNF-H released into the extracellular compartment thus analysis of CSF and serum neurofilaments levels may provide a valuable tool to determine the extent of axonal injury in MS patients [12].

Our study included 40 patients along with 40 healthy controls. This study included more females than males with 25 females and 15 male with a ratio 1.6 : 1. These results are similar with results of epidemiologic study about MS which have shown high prevalence of MS in young adults and females suggesting that hormones may play a significant role in determining susceptibility to MS [13].

Patients with FDE or RRMS were younger than those with SPMS or PPMS and had shorter duration disease than them. Accordingly, the median EDSS was higher in progressive forms of MS compared to RRMS. The BMI is also higher in the progressive groups than the other ones.These results are in accordance with a previous study as regard age, disease duration and EDSS [14].

In our study there was highly statistically significant difference in the BMI between our patients with the highest body mass index were for PPMS patient group (26.5) and the lowest BMI were for FDE patient group (22.5). The BMI was higher in the progressive group than relapsing remitting one.

The serum pNF-H was shown to be significantly higher in our MS patients compared with controls. Neurofilaments, a major cytoskeletal constituent of neuronal cells, can be released into the cerebrospinal fluid and serum due to axonal injury in different neurological diseases such as multiple sclerosis. This finding is consistent with a study found that the level of pNF-H was higher in patients with all subtypes of MS than control [6]. This result also in agreement with another study found that pNF-H levels were higher in MS patients in all disease stages compared to control [15]. Previous study Massouda and colleagues also found there was a highly statistically significant difference in the levels of pNF-H in patients with MS when compared with control group[16].

Our finding of a positive correlation between patient disability measured by EDSS and pNF-H protein levels is in agreement with a previous study[15] who found that p-NFH levels correlated with EDSS scores in patients with clinically isolated syndrome(CIS) and RRMS, the correlation was most prominent in RRMS during relapse. Also another study found a positive correlation between patient disability and pNF-H protein levels [17]. A previous study carried on the relation between pNF-H and EDSS showed that the level of pNF-H significantly increased with the progression of MS as measured by EDSS [16]. Axonal injury is recognized as the main pathological cause of progressive neurological deterioration in MS so that this positive correlation between pNF-H and disability validated this protein as a likely surrogate marker for axonal injury in MS patients [6].

We also demonstrated a positive correlation between pNF-H and the age of patients and we also found a positive correlation between pNF-H and the age of controls. Previous study described a weak correlation of pNf-H with age in patients with

MS while correlations for controls were not reported [18]. Also another study found correlations of pNf-H levels of disease groups with age were considerably stronger in their study and CSF levels of pNF-H increased with age in controls and CIS this effect was less pronounced in RRMS and absent in SPMS/PPMS [20].Increase in neurofilament with normal aging and in neurodegenerative disease due to declining phosphatase activities during normal aging leading to neurofilament hyperphosphorylation, and this may alter functions of NF. Hyperphosphorylation of NF in age-related neurodegenerative disorders has been attributed to activation of multiple protein kinases and reduced activity of protein phosphatases.These enzymatic changes have been implicated in promoting abnormal perikaryal NF accumulation, and defective axonal transport leading to neuronal cell death increasing its realease in CSF and serum.Therefore, raising phosphatase activity might reduce the hyperphosphorylation of cytoskeleton in aging and age-related neurodegenerative disorders. So pharmacologic approaches that elevate phophatase activity have been shown to have potential therapeutic effects in neurological disease models [19].

In our study there was significant difference in serum neurofilament levels between patients according to treatment type (treated on fingilomod or on interferon or patients not on modifying therapy) with the higher level of neurofilament in untreated group and the lower level of neurofilament in patients on fingilomod. Fingilomod is the first oral MS disease-modifying therapy, Fingolimod can cross the blood–brain barrier into the central nervous system to be phosphorylated to its active metabolite, fingolimod-P. Fingolimod-P then potentially interacts with sphingosine1- phosphate receptors which located on oligodendrocytes, astrocytes, neurons, and microglia, and also on vascular endothelial cells of the blood–brain barrier. So that Fingolimod can reduce demyelination and promote remyelination throught its direct effects in the CNS. Results from phase 3 trials of fingolimod found that the preservation of neural cells and axons observed preclinically may be related to the efficacy on brain atrophy outcomes observed in patients with MS [20].

We recommended that neurologist should consider adding use of pNF-H serum level to their routine laboratory tests in multiple sclerosis patients since it is significantly correlated with clinical and paraclinical parameters of the disease .

We recommend using of Fingilomod as DMTs because patients with lowest level of pNF-H are on Fingilomod treatment, it readily crosses the BBB, reduce demyelination and promote remyelination via direct effects in the CNS leading to CNS preservation effects.

We also recommend studies on pharmacologic approaches that elevate phophatase activity to reduce the hyperphosphorylation of cytoskeleton in aging and age-related neurodegenerative disorders, it will have a potential therapeutic effects in neurological disease models.

Conflict of Interest: There are no conflicts of interest .

Funding: No funding sources.

REFERENCES

1-Kobelt G, Thompson A, Berg J, Gannedah M, Eriksson J. New insights into the burden and costs of multiple sclerosis in Europe. Mult Scler J. 2017;23: 1123–1136.

2-Gresle MM, Butzkueven H, Shaw G. Neurofilament proteins as body fluid biomarkers of neurodegeneration in multiple sclerosis. Int. MS J. 2011;2011: 6- 13.

3- Bjartmar C and Trapp B. Axonal and neuronal degeneration in multiple sclerosis: mechanisms and functional consequences. Curr. Opin. Neurol.2001; 14: 271–278.

4- Sahraian MA and Eshaghi A . Role of MRI in diagnosis and treatment of multiple sclerosis. Clin. Neurol. Neurosurg.2010;112:609–615.

5- Zivadinov R and Leist TP.Clinical-magnetic resonance imaging correlations in multiple sclerosis. J. Neuroimaging.2005;15:10S–21S.

6- Miller DH, Grossman RI, Reingold SC, McFarland HF. The role of magnetic resonance techniques in understanding and managing multiple sclerosis. Brain. 1998;121(1):3–24

7-Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J. Neurol. Sci.2005;233:183–198.

8-Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis:2010 revision to the Mcdonald criteria. Ann Neurol. 2011;69:292-302.

9- Gresle MM, Shaw G, Jarrott B. Validation of a novel biomarker for acute axonal injury in experimental autoimmune encephalomyelitis. J Neurosci Res.2008;86:3548-55.

10- Boylan K, Yang C, Crook J,Overstreet K, Heckman M, Wang Y et al .Immunoreactivity of the phosphorylated axonal neurofilament H subunit (pNF-H) in blood of ALS model rodents and ALS patients: evaluation of blood pNF-h as a potential ALS biomarker. J Neurochem.2009;111:1182-91.

11-Coolidge F. Statistics a gentle introduction third edition.SAGE publication.2012;1-38.

12-Teunissen C, Dijkstra P, Polman C. Biological markers in CSF and blood for axonal degeneration in multiple sclerosis. Lancet Neurol.2005;4:32-41.
13-McCombe P,Greer J. Female reproductive issues in multiple sclerosis. Mult Scler.2013;19:392-402.
14-Gresle M, Liu Y, Dagley L, Haartsen J, Pearson F.Serum phosphorylated neurofilament- heavy chain levels in multiple sclerosis patients. J Neurol Neurosurg Psychiatry.2014;85:1209-1213.
15-Kuhle J, Leppert D, Petzold A, Regenitter A, Schindler C, Mehling M. Neurofilament heavy chain in CSF correlates with relapse and disability in multiple sclerosis. J Neuroimmunol 2011;220:114-119
16- Massouda H, Saif E, Khattabb S, Saed G. Neurofilament as a bio marker of axonal loss in multiple sclerosis. Sci J Al-Azhar Med Fac, Girl 2017;1:63-68.
17-Fialova LI, Bartos A, Svarcova J, Zimova D, KotoucovaJ, Malbohan I. Serum and cerebrospinal fluid light neurofilaments and antibodies against them in clinically isolated syndrome and multiple sclerosis. J Neuroimmunol.2013;262:113-120.
18-Teunissen C, Iacobaeus E, Khademi M, Brundin L, Norgren N, Koel-Simmelink M, et al. Combination of CSF N-acetylaspartate and neurofilament in multiple sclerosis. Neurology 2009;72:1322-1329.
19- Veeranna, Yang DS, Lee JH, Vinod KY, Stavrides P, Amin ND, Pant HC, et al. Declining phosphatases underlie aging-related hyperphosphorylation of neurofilaments. Neurobiol Aging. 2011;32:2016- 29.
20- Groves A, Kihara Y, Chun J. Fingolimod: direct CNS effects of sphingosine1-phosphate (S1P) receptor modulation and implication in multiple sclerosis therapy.J Neurol Sci 2013;328:9-18

How to cite
Elmasry, D., AbdElghaffar, A., Elebeary, M., Aidaros, M., Shawky, N. SERUM PHOSPHORYLATED NEUROFILAMENT-HEAVY CHAIN LEVELS IN MULTIPLE SCLEROSIS PATIENTS. Zagazig University Medical Journal, 2022; (45-51): -. doi: 10.21608/zumj.2019.15900.1424