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Blood biomarkers of neuronal degeneration in patients with relapsing remitting multiple sclerosis and the impact of treatment with interferon beta on their level.

Rasha Soliman[1], Mona Hussein[2], Noha A Doudar[3], Hend M Abdelhamid[4] & Amira Mohammed[5]

1. Professor of Neurology, Neurology department, Beni-Suef, Beni-Suef University, Egypt
2. Assistant Professor of Neurology, Neurology department, Beni-Suef University, Beni-Suef, Egypt
3. Assistant Professor of Clinical and Chemical pathology, Clinical and Chemical pathology department, Beni-Suef University, Beni-Suef, Egypt
4. Assistant lecturer of Neurology, Neurology department, Beni-Suef University, Beni-Suef, Egypt
5. Lecturer of Neurology, Neurology department, Beni-Suef University, Beni-Suef, Egypt

Corresponding Author: Mona Hussein, MD.
Email: ameeraali201@gmail.com

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Abstract

Objectives: Strong evidence theorizes that neurodegeneration and axonal loss in multiple sclerosis (MS) may occur independently of or may even be the cause of the demyelination in MS. The aim of this work was to assess the extent of neuronal degeneration and axonal damage in patients with relapsing remitting multiple sclerosis (RRMS) through measuring the serum level of S100B and total tau, and to evaluate the impact of treatment with interferon beta on their level.

Methods: The study was conducted on 30 patients fulfilled the McDonald's criteria for diagnosis of RRMS, and 30 healthy controls. All included patients were subjected to neurological and cognitive assessment in addition to assessment of fatigue. Serum S100B and total tau were measured for all included patients (before and 6 months after treatment with interferon beta) and for controls as well.

Results: MS patients had significantly higher serum level of S100B than controls (P-value= <0.001), but there was no statistically significant difference between MS patients and controls in serum Tau (P-value= 0.77). There was a statistically significant decrease in S100B serum level in MS patients after 6 months of treatment with interferon beta (P-value <0.001), but there was no significant change in serum Tau (P-value 0. 098).

Conclusion: RRMS patients had a significantly higher S100B serum levels in comparison to healthy controls. Treatment with interferon beta significantly improved neurodegeneration in patients with RRMS.

Key words: Multiple sclerosis; EDSS; S100B; Tau; INF-B.

Introduction

The burden of MS has increased markedly during the recent years. The increase was estimated to be 30% from 2013 to 2020 with approximately 2.8 million MS patients according to 2020 epidemiology survey. [1] The challenging part of MS management is related to affecting the young population. MS is considered the most common cause of neurologic disability in young population.[2]

MS was believed to be an inflammatory demyelinating disease. In early stages of the disease, it is characterized by infiltration and activation of T cells and accumulation of monocyte-derived macrophages, which promote destruction of the myelin sheath leading to the formation of focal demyelinated lesions. [3] Axonal degeneration also occurs due to loss of oligodendrocytes.[4] Most MS patients start their disease by being relapsing remitting then can convert to secondary progressive MS. The repeated relapses and inflammation were thought to be the main reason for accumulating disability and degeneration.[5] There was a hypothesis of a two-stage disease, inflammatory phase then neurodegeneration. However, lots of pathologic and neuroimaging markers proved that the neurodegeneration start at very early of the disease course. [6]

Several markers been used to demonstrate MS activity and axonal damage. In previous studies, total Tau protein (tTau) and S100B protein were measured to monitor the extent of neuronal damage in patients with multiple sclerosis. [7] Tau protein is a phosphorylated microtubular protein which has a significant role in stabilizing the microtubules and maintaining structural polarization of neurons. [8] S100B is a small Ca^{2+}-binding protein which promotes neuronal proliferation, oligodendrocyte differentiation and assembly of cytoskeleton components.[9]

The aim of this work was to assess the extent of neuronal degeneration and axonal damage in patients with relapsing remitting multiple sclerosis (RRMS) through measuring the serum level of S100B and total tau, and to evaluate the impact of treatment with interferon beta on their level.

Methods

Study design

This case-control study that was conducted on 30 patients diagnosed with RRMS (before and 6 months after treatment with interferon beta) and 30 healthy controls. The patients were recruited from the Neurology clinic, Beni-Suef University Hospital, in the period from January 2020 to October 2022.

Eligibility criteria

This case-control study included patients fulfilling “McDonald’s criteria 2017” for diagnosis of relapsing remitting multiple sclerosis (RRMS) (during remission state at least one month after the last dose of intravenous methyl prednisolone (IVMP))[10] The included patients should be naive for disease modifying therapy and eligible for treatment with interferon beta. Age range was between 18 and 45 years.

The following patients were excluded from the study: patients with a history of any associated autoimmune disease, CNS inflammatory or infectious disorder, traumatic brain injury, or neurodegenerative disease, patients with concomitant medical or metabolic illness known to affect S100B or Tau, patients with contraindication for treatment with interferon beta or those who developed serious complications from treatment with interferon beta.

Clinical assessment

History was taken from the included RRMS patients focusing on disease duration and total number of relapses. Assessment of neurological disability was done using Expanded Disability Status

Scale (EDSS) [11]. Assessment of fatigue was done using Modified fatigue impact scale (MFIS).[12]

Cognitive assessment of the included RRMS patients and controls was done using the following psychometric tests: Paired Associate Learning test (PALT) (for assessment of verbal memory) [13], Benton Visual Retention test (BVRT) (for assessment of visual perceptual, visual memory, and visuoconstructive abilities)[14], and Paced Auditory Serial Addition Test (PASAT) (for assessment of attention and working memory)[15]

Radiological assessment:

Magnetic resonance imaging (MRI) on brain, cervical and dorsal segments of spinal cord were performed for all the included RRMS patients to detect the site and number of MS plaques and to exclude other structural brain or spinal cord lesions. The scan was done by a 1.5 Tesla Simens scanner, Germany. The following sequences were taken: T2- weighted images (axial, coronal), Fluid attenuated inversion recovery (FLAIR), T1- weighted images (axial, sagittal), and Gadolinium enhanced T1-weighted axial and sagittal images.

Laboratory assessment

Serum S100B and total tau was measured for all included patients (before and 6 months after treatment with interferon beta) and for controls as well. This was applied at clinical pathology department, Beni-suef university hospital.

Five ml of peripheral venous blood samples were collected from the patients with MS and healthy controls into sterile polyprophylene tubes. The serum was separated after the blood samples were allowed to be clotted and centrifuged at 3500 rpm for 10- 20 min and separated into aliquots. Then the samples were stored frozen at −80°C freezer until use for measuring Tau and S100B. The samples were consecutively identified by number to guarantee confidentiality. Measuring S100B and Tau serum level by enzyme-linked immunosorbent assay (ELISA)

ELISA kits for the quantitative level of Human S100B and Tau in the sample (SinoGeneClon Biotech Co.,Ltd Cat No: SG-11708 &2SG-16522, respectively) ,adopt purified S100B / Tau antibody to coat microtiter plate, make solid-phase antibody, then add S100B / Tau to wells, Combine S100B /Tau antibody with labeled HRP to form antibody-antigen -enzyme antibody complex, after washing completely, add TMB substrate solution, TMB substrate becomes blue color at HRP enzyme catalyzed, reaction is terminated by the addition of a stop solution and the color change is measured at a wavelength of 450 nm. The concentration of S100B or Tau in the samples is then determined by comparing the O.D. of the samples to the standard curve. Specific dilutions, incubation periods and temperature are applied according to manufacturer protocol.

Sampling

The sample size was calculated using Epi info, version 3.5.1, 2008. Based on the 35.9/100,000 prevalence rate of multiple sclerosis [1], a total sample size of at least 16 patients was required to achieve a confidence level of 99.9%.

Statistical analysis:

IBM SPSS Version 25 was used to analyze the data. Sex was expressed as number (%). Age was expressed as mean (SD), while years of education, disease duration, total number of relapses, EDSS, MFIS, psychometric tests, S100B, Tau, and MRI lesion load were expressed as median (IQR). Chi-squared test was used for comparison between RRMS patients and control groups in sex. Independent sample t-test was used for comparison between RRMS patients and control groups in age, whereas *Mann-Whitney test* for comparison between RRMS patients and control groups in years of education, psychometric tests, S100B, and Tau. *Wilcoxon* test was used for comparison between S100B and Tau in RRMS patients before and 6 months after treatment with

interferon beta. The Spearman correlation coefficient (r) was used to correlate baseline S100B and Tau with years of education, disease duration, total number of relapses, EDSS, MFIS, psychometric tests, and MRI lesion load. The probability/significance value (P-value) ≤ 0.05 was considered statistically significant.

Results

Demographics, clinical, and radiological characteristics of MS patients

This case-control study was conducted on 30 RRMS patients and 30 healthy controls. There was no statistically significant difference between RRMS patients and controls regarding age, sex or years of education (P-value = 0.278, 0. 297, 0. 239 respectively) (Table 1).

Regarding the clinical characteristics of RRMS patients, the median value for disease duration was 4 (IQR=5) months, for the total number of relapses was 3 (IQR=2), for EDSS was 3 (IQR=1.5), and for MFIS was 7 (IQR=4.5) (Table 1).

Cognitive assessment of RRMS patients and controls revealed that RRMS patients had significantly lower scores in PALT, BVRT, and PASAT in comparison to controls (P-value = <0.001, 0.039, <0.001 respectively) (Table 1).

Regarding MRI lesion load in RRMS patients, the median value for the total number of MS lesions was 5 (IQR= 3) (Table 1).

Serum S100B and Tau in MS patients and controls

The median value for baseline S100B in RRMS patients was 625.05mg/dl (IQR= 278.42) and the median value in controls was 234.6 mg/dl (IQR= 229.1). There was a statistically significant difference between RRMS patients and controls (P-value <0.001). Regarding baseline serum Tau, the median value in RRMS patients was 240.4mg/dl (IQR= 177.68) and the median value in controls was 273.4 mg/dl (IQR= 80.13). There was no statistically significant difference between RRMS patients and controls (P-value= 0. 717) (Table 1).

After 6 months of treating RRMS patients with interferon beta, there was a statistically significant decrease in serum S100B (P-value <0.001), but there was no statistically significant change in serum Tau (P-value 0. 098) (Table 2, Figure 1).

Correlations between both baseline serum S100B and Tau in RRMS patients, and age, clinical, and radiological characteristics

There were statistically significant positive correlations between baseline serum S100B and both EDSS (r. coef. = 0.437, P-value= 0.016) and the total number of MS lesions (r. coef. = 0.497, P-value= 0.005). There were no statistically significant correlations between baseline serum S100B or Tau and either age, disease duration, total number of relapses, MFIS, or the psychometric tests (Table 3, Figure 2, 3).

Table (1**):** Demographics, clinical, laboratory, and radiological characteristics of RRMS patients

		Patients (n=30)	Controls (n=30)	P-value
Age in years [Mean (SD)]		34.16 (8.34)	32.06 (6.39)	0.278
Sex	Male [n (%)]	11 (36.7%)	15 (50.0%)	0.297
	Female [n (%)]	19 (63.3%)	15 (63.3%)	
Years of education [Median (IQR)]		12 (4)	12.5 (5.5)	0.239

Disease duration in months [Median (IQR)]		4 (5)		
Total number of relapses [Median (IQR)]		3 (2)		
EDSS [Median (IQR)]		3 (1.5)		
MFIS [Median (IQR)]		7 (4.5)		
Psychometric tests [Median (IQR)]	PALT	11.75 (7**)**	17.25 (3.00)	<0.001*
	BVRT	19 (5.25)	23 (13**)**	0.039*
	PASAT	5.5 (3)	8 (2)	<0.001*
Baseline serum S100B in mg/dl [Median (IQR)]		625.05(278.42)	234.6 (229.1)	<0.001*
Baseline serum Tau in mg/dl [Median (IQR)]		240.4 (177.68)	273.4 (80.13)	0. 717
MRI lesion load [Median (IQR)]		5 (3**)**		

BVRT: Benton Visual Retention test, EDSS: Expanded Disability Status Scale, MFIS: Modified Fatigue Impact Scale, MRI: Magnetic resonance imaging, PALT: Paired Associate Learning test, PASAT: Paced Auditory Serial Addition Test *P-value ≤ 0.05 (significant)

Table (2): Serum S100B and Tau in RRMS patients before and 6 months after treatment with interferon beta

	At baseline	After 6 months	P-values
Serum S100B in mg/dl [Median (IQR)]	625.05(278.42)	378.65 (331.98)	<0.001*
Serum Tau in mg/dl [Median (IQR)]	240.4 (177.68)	186.6 (207.7)	0.098

*P value ≤ 0.05 (significant).

Table (3): Correlations between both baseline serum S100B and Tau in RRMS patients, and age, clinical, and radiological characteristics

	Baseline serum S100B		Baseline serum Tau	
	(r) coef.	P-value	(r) coef.	P-value
Age in years	0.336	0.07	0.031	0.872
Disease duration in months	0.194	0.305	0.018	0.924
Total number of relapses	0.116	0.541	-0.115	0.547
EDSS	0.437	0. 016*	0.189	0.316
MFIS	0.243	0 .195	0.239	0.203

Psychometric tests	PALT	-0.040	0.833	0.020	0.916
	BVRT	0.219	0.246	-0.176	0.353
	PASAT	-0.148	0.435	0.031	0.869
MRI lesion load		0.497	0.005*	0.286	0.125

BVRT: Benton Visual Retention test, EDSS: Expanded Disability Status Scale, MFIS: Modified Fatigue Impact Scale, MRI: Magnetic resonance imaging, PALT: Paired Associate Learning test, PASAT: Paced Auditory Serial Addition Test. (r) Spearman coefficient, *P-value ≤ 0.05 (significant)

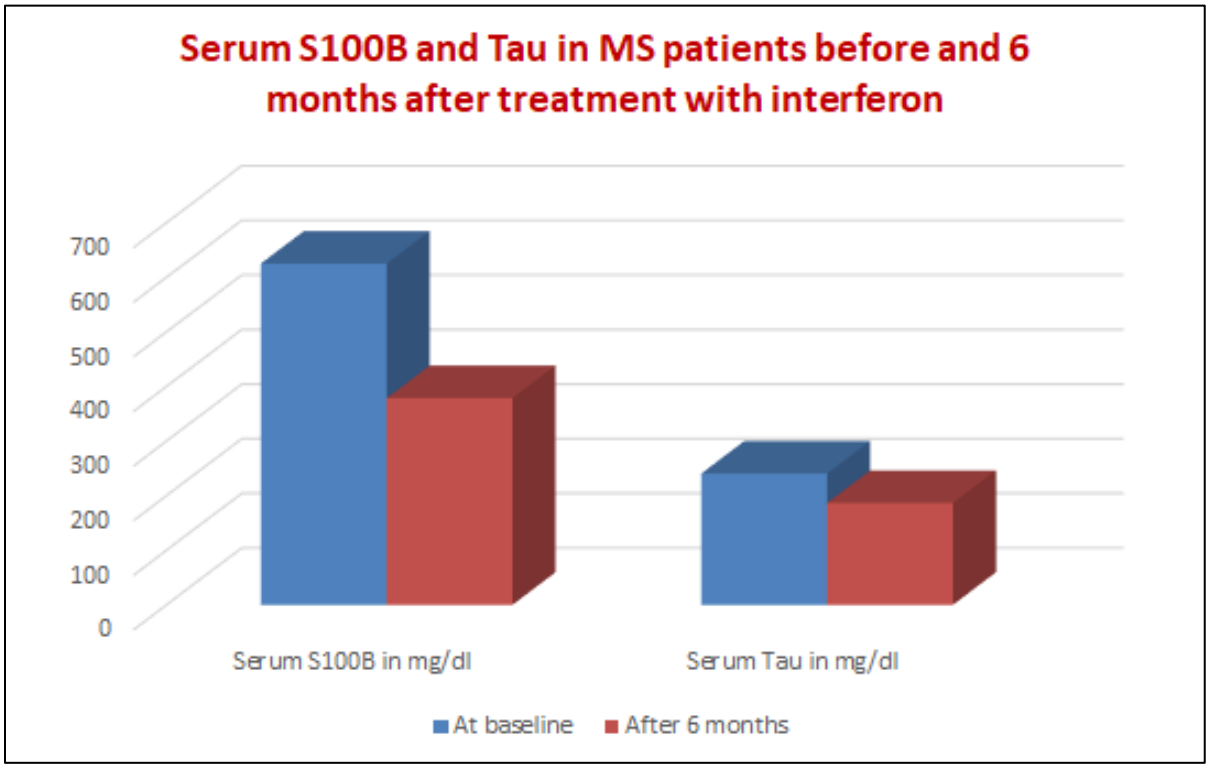

Figure (1): Serum S100B and Tau in MS patients before and 6 months after treatment with interferon beta

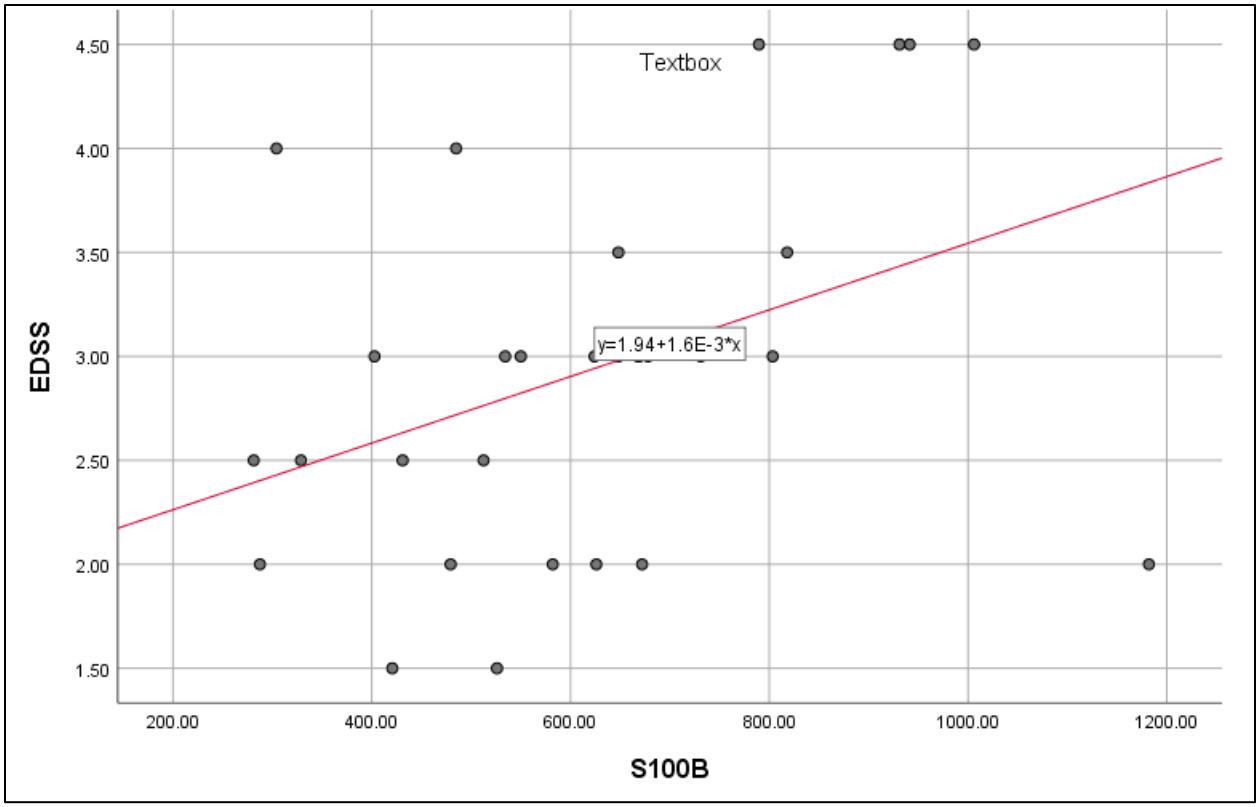

Figure (2): Correlation between baseline serum S100B and EDSS

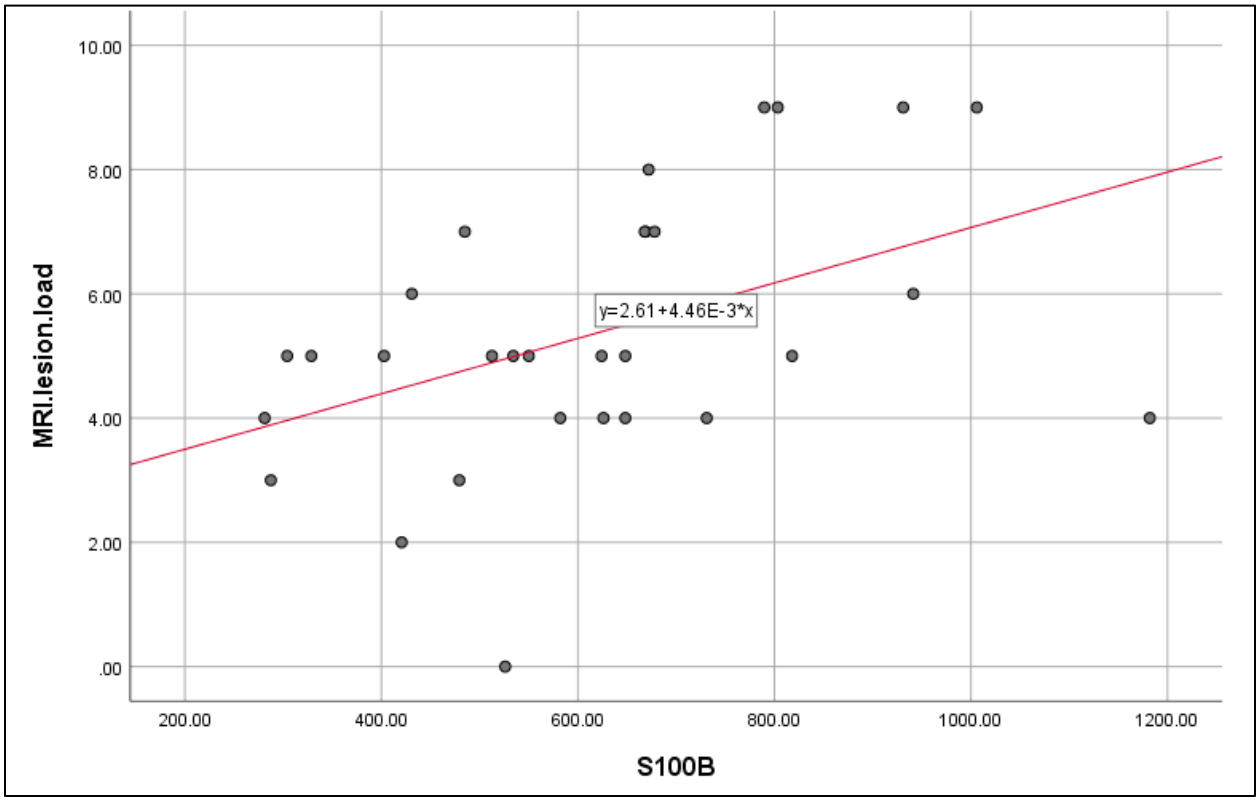

Figure (3): Correlation between baseline serum S100B and MRI lesion load

Discussion

Our study found that newly diagnosed patients with RRMS had significantly lower scores in PALT, BVRT, and PASAT psychometric tests compared to controls. The level of S100B was significantly higher in the newly diagnosed RRMS patients compared to healthy controls who were matched with them in age and sex. There was a positive correlation between S100B and each of EDSS and the MRI lesion load for MS patients. Treatment with Beta Interferon for 6 months markedly decreased the level of S100B. As regards the serum level of Tau, there was no difference between the MS patients and the control groups. In addition, there was no impact of the treatment on serum Tau level. There was no correlation between serum Tau and any of the clinical or imaging characteristics of MS patients.

Neurodegeneration was though to be a pathophysiologic process that occurs to the brain axons after long time of recurrent demyelination events. Recently, it has proven that neurodegeneration can happen in all the stages of the MS disease.[16, 17] Therefore, the neurodegeneration markers can play a major role in MS diagnosis and management.[18] S100B is a protein expressed by astrocytes and help in differentiation of the oligodendrocytes if secreted in low amounts.[9] Subsequent to the neuronal injury, S100B is released in toxic amounts which can have a detrimental impact on the oligodendrocytes and the axons.[19, 20]

Rejdak et al measured the serum and CSF levels of S100B for RRMS patients having a clinical relapse after a median of 3 years disease duration. S100B was higher in RRMS patients compared to controls.[21] Another study examined the S100B serum level in stable RRMS patients away from any relapse and found similar results.[22] What distinguishes our study that S100B serum level was found to be elevated in RRMS patients at the time of diagnosis which means the very early phase of the disease. The increase in S100B level was associated with disruption of the blood-brain barrier (BBB)[23-25] which could be related to the inflammatory activity in the very early stages of MS.[26] One study didn't find a significant difference in S100B between MS patients and control groups and explained that by recruiting MS patients with stable course of disease.[27] another observation that in this study, the controls had a higher age than the patients which may have compensated the expected high level of S100B in MS patients as S100B positively correlates with age.[28]

Similar to our analysis, there was a positive correlation between S100B levels and the clinical disability of MS patients in the same mentioned study. The patients with higher disability scores had higher S100B levels. [27] In addition, our data was the first to reveal a significant positive correlation between serum level of S100B and MRI lesion load which is explained by the association of each of S100B and MRI lesions burden with the MS inflammatory activity.[29] In a study included patients with PPMS, there was no association between S100B and any of EDSS or the MRI lesion load.[30]

As regards studying the impact of treatment on S100B serum level, O'Connell et al conducted a study on 37 MS patients; 11 patients were treated with interferon beta, 14 treated with natalizumab and 12 were drug naive. This study showed that patients treated with natalizumab and interferons showed significantly reduced S100B levels than drug naive MS patients.[31] However, baseline measurements of S100B before treatment were not available and also the duration of treatment with beta interferon was unknown. Fifty patients with PPMS were recruited in a trial of IFNβ-1a and were assessed over a study period of 2 years.[30] Serum S100B was measured at the beginning of the study and all over the two years of treatment. There was no change in S100B in PPMS

patients who received beta interferon or even the patients who received placebo. That confirms the association of S100B with the inflammatory activity more than the progression in MS. To our knowledge, our study was the first to prove that S100B declines markedly after receiving beta interferon treatment for 6 months in newly diagnosed RRMS patients. Beta interferon treatment reduces MS inflammatory activity by exerting immunomodulatory properties and maintaining the BBB integrity[32] which may contribute to the decline of S100B in our RRMS patients.

There was no difference in serum Tau level between MS patients and the control group in our study. Also, the serum level of Tau was not affected by the beta interferon treatment. The authors of a previous study measured the level of Tau before and after the treatment of mitoxantrone and didn't find any impact of the treatment and concluded that they don't appreciate serum level of Tau as a marker of MS activity.[22] However, the same study found a higher level of CSF Tau in MS patients compared to control. Tau protein concentration in serum is significantly lower than that in CSF.[33] This may provide an explanation for the lack of a significant difference between MS patients and controls regarding the serum Tau level.

Cognitive impairment is a clinical sign of the neurodegenerative process along the course of MS disease. It was thought to be more common and severe in patients with progressive MS.[34, 35] However, our data showed that RRMS patients had lowers scores than controls in PALT, BVRT, and PASAT neuropsychology tests. The median of EDSS for our patients was 3. Another study was concerned with the cognitive assessment in MS patients recruited 92 RRMS patients who had very mild clinical disability with median EDSS 1.[36] About 51% of those MS patients were found to have cognitive impairment. They have lower scores than the healthy control group in a number of tests like: California Verbal Learning Test (CVLT) and Brief Visuospatial Memory Test (BVMT). Additionally, the diseased group had lower scores on PASAT test similarly to our study. Another study used different set of tests and had the same conclusion that cognitive impairment can occur in the early stages of RRMS and mainly include the attention and the process speed.[37] In our study, there was no correlation between serum level of S100B or Tau and cognitive tests scores. Future studies should be held to confirm this finding.

Our study confirms the significance of the S100B biomarker in the diagnosis and clinical course of MS disease especially RRMS subset of patients. Additionally, it was the first study to investigate the impact of interferon beta on the serum level of both S100B and total tau in newly diagnoses RRMS patients.

Our study had some limitations. Firstly, the small sample size. Secondly, CSF S100B and total tau were not measured in our study. Thirdly, we didn't assess the brain atrophic changes in the included MS patients through structural MRI volumetric studies.

Conclusion:

RRMS patients had a significantly higher S100B serum levels in comparison to healthy controls. S100B serum level was positively correlated with disability and MRI brain lesion load but not with disease duration, total number of relapses, fatigue or cognitive function. S100B serum level in RRMS patients showed a significant decline after 6 months of treatment with INF-B.

Declarations:

Authors report that the content has not been published or submitted for publication elsewhere.

Competing interests

Authors have no competing interest.

Availability of data and materials

Authors report that the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethics approval and Consent to participate

A written informed consent was obtained from all included participants in this study or their first-degree relatives. The study was performed in accordance with the Declaration of Helsinki. The study was ethically approved by Faculty of Medicine, Beni-Suef University Research Ethical Committee (FM-BSU-REC). The ethical approval number was FWA00015574.

Authors' Contribution

RS participated in study design, interpretation of data, and helped to draft manuscript. MH participated in study design, analysis and interpretation of data, and helped to draft manuscript. ND participated in laboratory workup, interpretation of data, and helped to draft manuscript. HA participated in interpretation of data and helped to draft manuscript. AM participated in collection of data and helped to draft manuscript. All authors read and approved the final manuscript.

Consent for publication

Not applicable

Acknowledgements

Not applicable

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