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| RESEARCH ARTICLE

Relationship between Serum Glial Fibrillary Acidic Protein and Neurogranin Levels and Cognition in Multiple Sclerosis

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| ABSTRACT
Multiple sclerosis is an inflammatory neurodegenerative disorder, and cognitive problems occur in the early and late phases of the disease. The purpose of this study was to investigate the relationship between serum glial fibrillary acidic protein and neurogranin levels and cognition in patients with multiple sclerosis (MS). Twenty-three patients and 25 healthy controls were included in the study. Serum glial fibrillary acidic protein (GFAP) and neurogranin (NRGN) levels were determined on blood samples from patients and controls. Disease duration and EDSS scores of patients were recorded, and the Montreal Cognitive Assessment (MOCA) scale was used for cognitive assessment. There was no statistically significant difference between the two groups in terms of serum NRGN and GFAP levels. MOCA scores were lower in the patient group than in the healthy control group. No statistically significant correlation was found between NRGN and GFAP serum levels and MOCA scores. Our study showed that there was no statistically significant association between serum NRGN and GFAP levels and cognition in MS patients. This study is the first to examine serum GFAP and NRGN levels in the context of cognition in MS.

| KEYWORDS
Multiple sclerosis, Cognition, Neurogranin, Glial fibrillary acidic protein

| ARTICLE INFORMATION
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1. Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating, and neurodegenerative disease of the central nervous system (CNS), and both neurodegeneration and inflammatory mechanisms have crucial roles in the pathogenesis of the disease. Because this disease may lead to disability, especially in the young adult population, early and correct diagnosis is important to prevent disability, as it allows appropriate and early treatment. MS may have many symptoms, which can significantly affect the quality of life and lead to disability. Cognitive impairment (CI) may also occur in the course of MS and may lead to disability (Giazkoulidou,2019). CI in MS may arise in the earliest stages of the disease and cause serious limitations in the quality of life, work life, and family life. Various tests are used to evaluate cognitive functions in MS, but few serum biomarkers have a clear association with cognitive functions. In recent years, evidence has emerged of biomarkers being associated with neurodegeneration, especially neurofilament (NfL) light chain, which can be used in treatment and disease activity follow-ups in MS. However, the economic cost of testing for this biomarker is high and, therefore, difficult to apply in routine clinical practice. It is thus important to search for more easily accessible and economically viable biomarkers.

Evaluations with neuropsychological tests show the presence of cognitive dysfunction in most patients with MS. However, CI can only be detected in a minority of patients during clinical visits. Cognitive dysfunction in MS is an important factor that negatively affects a person's activities of daily living and work efficiency, regardless of the physical disability caused by the disease. However, the physical disability caused by the disease seems more relevant to the patient and the physician, and cognitive dysfunction is

therefore ignored owing to the inadequacy of the classical scales used in clinical visits in this area and the fact that it often cannot be adequately defined. In this respect, it would be clinically important to have a biomarker that allows easy detection of cognitive dysfunction or that indicates that cognitive dysfunction may be present. Serum levels of glial fibrillary acidic protein (GFAP) and neurogranin (NRGN), two biomarkers that may be associated with neurodegeneration, have also been studied in patients with MS, but few clinical studies have been described. GFAP is the main intermediate cytoskeleton protein of astrocytes. It is a widely studied biomarker used to detect CNS injury, especially traumatic brain injury(Wang,2018). During astrocyte activation, it is released into the intercellular space and cerebrospinal fluid (CSF). Another biomarker, NRGN, a postsynaptic protein, is enriched in dendritic spines, and NRGN levels are high in mild CI (MCI) and Alzheimer's disease (AD)(Tahami Monfared,2022 and Blennow,2018). The biomarker role of these two molecules has been evaluated in many studies on many different subjects.

CI may be independent of other symptoms of MS and may worsen independently of other symptoms or precede physical disability, which may impair the quality of life. The presence of a biomarker that may indicate CI in MS may reveal more appropriate treatment options for these patients. In this respect, a correlation between serum GFAP and NRGN levels with disease activity, severity, and cognition may allow the use of these biomarkers in clinical practice.

2. Material and Methods

A total of 23 patients and 25 healthy controls were included in the study. Informed consent was obtained from the patient and control groups. Ethics approval was obtained from the XXXX University Non-interventional Research Ethics Committee. Patients with MS registered in the XXXX University Faculty of Medicine, Department of Neurology, were included in the study following the Mc Donalds 2017 revision. Serum NRGN and GFAP levels from blood samples taken and stored during routine examinations were determined using ELISA tests in the biochemistry laboratory. The Montreal Cognitive Assessment (MOCA) scale was used for cognitive assessment during routine examinations. In addition, routine laboratory tests of patients and healthy controls were added to the data processing system and recorded.

Statistical tests were performed using IBM SPSS 20, and $p < 0.05$ was considered statistically significant. Continuous variables were presented as mean standard deviation. Categorical variables were expressed as ratios. Student's t-test was used to test for differences in continuous variables, and the Chi-square test was used for categorical values. Relationships between serum biomarker levels and other parameters were examined in all participants using Spearman's correlation.

3. Results

There was no statistically significant difference between the groups in terms of age ($p = 0.07$) or gender ($p = 1.00$). There was no statistically significant difference between the two groups in terms of serum GFAP ($p = 0.107$) or NRGN ($p = 0.065$) levels. MOCA scores were statistically significantly lower in the patient group than in the healthy control group ($p = 0.016$). The mean duration of the disease was 7.87 ± 5.73 (Table 1). There was a statistically significant correlation between disease duration and MOCA scores ($p = 0.001$) and EDSS ($p = 0.009$) scores. No correlation was found between EDSS and MOCA scores and serum NRGN and GFAP levels (Table 2).

Table 1 – Characteristics of the patient and control groups

		Patient (n = 23)	**Control (n = 25)**	**p**
Gender	Female	19 (82.6%)	20 (80%)	1.000
	Male	4 (17.4%)	5 (20%)	
Age	Mean ± SD	38.6 ± 10.8	33.2 ± 9.6	0.070
GFAP	Mean ± SD	3.71 ± 1.89	2.83 ± 1.13	0.107
	Median [25-75th percentile]	2.74 [2.11 – 5.15]	2.48 [1.94 – 3.96]	
NRGN	Mean ± SD	159.42 ± 91.03	189.73 ± 84.07	0.065
	Median [25-75th percentile]	120.18 [95.25 – 231.62]	164.32 [120.76 – 234.64]	
EDSS	Mean ± SD	2.65 ± 2.12	-	-
	Median [25-75th percentile]	2 [1 – 4]	-	
Disease duration	Mean ± SD	7.87 ± 5.73	-	-
	Median [25-75th percentile]	6 [4 – 12]	-	
MOCA	Mean ± SD	26.39 ± 3.58	29.2 ± 0.91	0.016
	Median [25-75th percentile]	27 [23- 30]	29 [28,5 – 30]	

Table 2 – Correlations between the patient and control groups

	Patient		Control	
	Correlation Coefficient	**p**	**Correlation Coefficient**	**p**
GFAP - NRGN	-0.053	0.809	0.088	0.675
Disease duration – EDSS	0.530	0.009	-	-
Disease duration – GFAP	0.037	0.868	-	-
Disease duration – NRGN	0.104	0.638	-	-
Disease duration – MOCA	-0.649	0.001	-	-
EDSS – GFAP	0.066	0.765	-	-
EDSS – NRGN	0.056	0.799	-	-
EDSS – MOCA	-0.681	< 0.001	-	-
GFAP – MOCA	-0.039	0.858	-0.077	0.715
Neurogranin – MOCA	-0.082	0.710	0.172	0.410

4. Discussion

MS is an inflammatory degenerative disease that can cause serious disability in adulthood. CI frequently appears in the course of the disease and can cause serious problems in social life, at work, and during education. The deterioration in cognitive functions is evident in the later stages of the disease, but the effects may also begin in early-stage cases when the first clinical findings appear. CI in patients with MS negatively affects activities of daily life, and the prevalence of cognitive dysfunction ranges from 40% to 70% (DeLuca,2015). Cognitive problems in patients with MS can be revealed both during routine examination and with detailed cognitive tests. However, specific biomarkers that can reveal these cognitive problems are not currently available, but some studies on this subject have revealed promising evidence. For example, it has been suggested that CSF Tau levels at the time of diagnosis can provide important information on cognitive function and its follow-up in MS (Virgilio,2022). In another study, Rademacher et al. suggested that NfL light chain and vitamin D can be used as promising biomarkers to monitor CI in MS (Rademacher,2023). Martinez et al. suggested that the cognitive functions of patients with MS and positive lipid-specific oligoclonal IgM bands in the CSF are worse than those with negative IgM bands (Coll-Martinez,2022). Considering the issue from a broader perspective, Brummer et al. suggested that the combined use of serum and imaging biomarkers may be more valuable in detecting CI in MS (Brummer,2022). In our study, we investigated the relationship between GFAP and NRGN blood levels and cognition in patients with MS but did not find a statistically significant correlation.

NRGN is a postsynaptic protein expressed in the neocortex, amygdala, caudate nucleus, putamen, and hippocampus and is found in the human brain in cortical areas and layers II-IV in the cerebral cortex. Because NRGN is a small protein, it easily crosses a damaged blood-brain barrier. Studies on NRGN levels and blood-brain barrier damage have shown that high NRGN levels are associated with traumatic brain injury (Yang,2015). Serum NRGN levels are also significantly higher in patients diagnosed with acute spontaneous intracranial hemorrhage (Çevik,2019). According to these findings, serum NRGN values may be a useful biomarker in the preliminary diagnosis of intracranial hemorrhage. In addition, the relationship between NRGN levels and cognition has been studied especially regarding AD. For example, Xue et al. suggested that CSF NRGN levels could be used as a biomarker for AD (Xue,2020). In another study, it was shown that CSF NRGN levels were negatively correlated with mini-mental scale scores in patients with AD (Fan,2021). It has also been suggested that NRGN levels could be used as a biomarker in other neurological and mental diseases, such as Parkinson's disease and schizophrenia (Xiang,2020). Although a relationship between cognition and NRGN levels has been described in the literature, we could not detect a significant correlation between MOCA scores and serum NRGN levels in patients with MS in our study.

GFAP is the major intermediate filament protein of mature astrocytes. One of the key events in astrocyte differentiation is the initiation of GFAP expression (McCall,1996). Immature astrocytes initially secrete vimentin, whereas mature astrocytes secrete GFAP (Dahl,1981). Therefore, GFAP is considered an astrocyte maturation marker (Gomes,1999). GFAP plays a role in neuronal-glial interaction (McCall,1996), and thus, changes in GFAP levels may result in the disruption of neuron-neuron and neuron-glia connections. It has been suggested that plasma GFAP levels can be used as a biomarker for Alzheimer's pathology (Benedet,2021). In some studies, elevated serum GFAP levels have been associated with CI (Gonzales,2022). In addition, Oeckl et al. suggested that serum GFAP levels can be used to distinguish MCI from dementia and in the differential diagnosis of some types of dementia (Oeckl,2022). In another study, Chatterjee et al. showed that serum GFAP levels are higher in cognitively normal individuals who are at higher risk of developing AD (Chatterjee,2021). Changes in GFAP levels in saliva may be associated with cognitive disorders (Katsipis,2021). Asken et al. have suggested that plasma GFAP levels may be more sensitive than plasma NfL levels to changes in white matter and cognitive functions (Asken,2022). A recently published article suggested that serum GFAP levels could be used as a prognostic biomarker for future progression in MS patients independent of relapse activity (Meier,2023). However, a recent

study showed that elevated serum NfL levels correlate with CI in patients with active progressive MS but not with serum GFAP levels (Barro,2023). Consistent with the results of this study, we did not find a significant relationship between serum GFAP levels and MOCA scores in patients with MS in our study.

5. Conclusion

Serum GFAP and NRGN levels have been investigated in many studies as biomarkers that correlate with cognitive functions. CI is also a common problem in patients with MS and affects the quality of life, and various biomarkers have been associated with CI in MS. To the best of our knowledge, our study is the first to examine serum GFAP and NRGN levels in the context of cognition in MS. In our study, we investigated the relationship between cognitive involvement in MS and serum GFAP and NRGN levels, but no significant correlation was found.

Our study has some limitations. First, the number of patients was small, which may prevent the generalization of our results. Second, detailed cognitive tests were not performed when evaluating patients. This may have caused us to evaluate cognitive functions inappropriately. Third, only serum levels of biomarkers were determined; we did not have the opportunity to determine CSF levels.

More comprehensive studies should be performed to provide more useful information on this subject. For example, studying these biomarkers in CSF as well as serum and performing more detailed cognitive tests with a larger number of patients will provide more valuable information.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

References

[1] Asken BM, VandeVrede L, Rojas JC, Fonseca C, Staffaroni AM, Elahi FM, Lindbergh CA, Apple AC, You M, Weiner-Light S, Brathaban N, Fernandes N, Boxer AL, Miller BL, Rosen HJ, Kramer JH and Casaletto KB. (2022). Lower White Matter Volume and Worse Executive Functioning Reflected in Higher Levels of Plasma GFAP among Older Adults with and Without Cognitive Impairment. J Int Neuropsychol Soc. 2022 Jul;28(6):588-599.

[2] Barro C, Healy BC, Saxena S, Glanz BI, Paul A, Polgar-Turcsanyi M, Guttmann CR, Bakshi R, Weiner HL and Chitnis T. (2023). Serum NfL but not GFAP predicts cognitive decline in active progressive multiple sclerosis patients. Mult Scler. 2023 Feb;29(2):206-211.

[3] Benedet AL, Milà-Alomà M, Vrillon A, Ashton NJ, Pascoal TA, and Lussier F. (2021). Translational Biomarkers in Aging and Dementia (TRIAD) study, Alzheimer's and Families (ALFA) study, and BioCogBank Paris Lariboisière cohort. Differences Between Plasma and Cerebrospinal Fluid Glial Fibrillary Acidic Protein Levels Across the Alzheimer Disease Continuum. JAMA Neurol. 2021 Dec 1;78(12):1471-1483.

[4] Blennow K and Zetterberg H. (2018). Biomarkers for Alzheimer's disease: current status and prospects for the future. J Intern Med. 2018 Dec;284(6):643-663.

[5] Brummer T, Muthuraman M, Steffen F, Uphaus T, Minch L, Person M, Zipp F, Groppa S, Bittner S and Fleischer V. (2022). Improved prediction of early cognitive impairment in multiple sclerosis combining blood and imaging biomarkers. Brain Commun. 2022 Jul 8;4(4):fcac153. doi: 10.1093/braincomms/fcac153. PMID: 35813883; PMCID: PMC9263885.

[6] Chatterjee P, Pedrini S, Stoops E, Goozee K, Villemagne VL, Asih PR, Verberk IMW, Dave P, Taddei K, Sohrabi HR, Zetterberg H, Blennow K, Teunissen CE, Vanderstichele HM, Martins RN. (2021). Plasma glial fibrillary acidic protein is elevated in cognitively normal older adults at risk of Alzheimer's disease. Transl Psychiatry. 2021 Jan 11;11(1):27.

[7] Coll-Martinez C, Quintana E, Buxó M, Salavedra-Pont J, Gasull-Vicens L, Quiroga-Varela A, Costa-Frossard L, Villar LM, Fernández-Díaz E, Gracia J, Aladro Y, Méndez-Burgos A, Cerezo M, Ramió-Torrentà L and Gich J. (2022) Oligoclonal IgM bands are a promising biomarker for long-term cognitive outcomes in multiple sclerosis. Mult Scler Relat Disord. 2022 Dec;68:104397.

[8] Çevik S, Özgenç MM, Güneyk A, Evran Ş, Akkaya E, Çalış F, Katar S, Soyalp C, Hanımoğlu H, Kaynar MY. (2019). NRGN, S100B and GFAP levels are significantly increased in patients with structural lesions resulting from mild traumatic brain injuries. Clin Neurol Neurosurg. 2019 Aug;183:105380.

[9] Dahl D. (1981) The vimentin-GFA protein transition in rat neuroglia cytoskeleton occurs at the time of myelination. J Neurosci Res 1981;6:741-748.

[10] DeLuca GC, Yates RL, Beale H, Morrow SA. (2015). Cognitive impairment in multiple sclerosis: clinical, radiologic and pathologic insights. Brain Pathol. 2015 Jan;25(1):79-98.

[11] Fan Y, Gao Y, Therriault J, Luo J, Ba M, Zhang H. (2021). Alzheimer's Disease Neuroimaging Initiative. The Effects of CSF Neurogranin and *APOE* ε4 on Cognition and Neuropathology in Mild Cognitive Impairment and Alzheimer's Disease. Front Aging Neurosci. 2021 Apr 27;13:667899.

[12] Giazkoulidou A, Messinis L, Nasios G. (2019). Cognitive functions and social cognition in multiple sclerosis: An overview. Hell J Nucl Med. 2019 Jan-Apr;22 Suppl:102-110. PMID: 30877728.

[13] Gomes FC, Paulin D, Moura Neto V. (1999). Glial fibrillary acidic protein (GFAP): modulation by growth factors and its implication in astrocyte differentiation. Braz J Med Biol Res 1999;32:619-631.

[14] Gonzales MM, Wiedner C, Wang CP, Liu Q, Bis JC, Li Z, Himali JJ, Ghosh S, Thomas EA, Parent DM, Kautz TF, Pase MP, Aparicio HJ, Djoussé L, Mukamal KJ, Psaty BM, Longstreth WT Jr, Mosley TH Jr, Gudnason V, Mbangdadji D, Lopez OL, Yaffe K, Sidney S, Bryan RN, Nasrallah IM, DeCarli CS, Beiser AS, Launer LJ, Fornage M, Tracy RP, Seshadri S, Satizabal CL. (2022). A population-based meta-analysis of circulating GFAP for cognition and dementia risk. Ann Clin Transl Neurol. 2022 Oct;9(10):1574-1585.

[15] Katsipis G, Tzekaki EE, Tsolaki M, Pantazaki AA. (2021). Salivary GFAP as a potential biomarker for diagnosis of mild cognitive impairment and Alzheimer's disease and its correlation with neuroinflammation and apoptosis. J Neuroimmunol. 2021 Dec 15;361:577744.

[16] McCall MA, Gregg RG, Behringer RR, Brenner M, Delaney CL, Galbreath EJ, et al. (1996). Targeted deletion in astrocyte intermediate filament (Gfap) alters neuronal physiology. Proc Natl Acad Sci 1996;93:6361-6366.

[17] Meier S, Willemse EA, Schaedelin S. (2023). Serum Glial Fibrillary Acidic Protein Compared With Neurofilament Light Chain as a Biomarker for Disease Progression in Multiple Sclerosis. *JAMA Neurol.* 2023;80(3):287–297.

[18] Oeckl P, Anderl-Straub S, Von Arnim CAF, Baldeiras I, Diehl-Schmid J, Grimmer T, Halbgebauer S, Kort AM, Lima M, Marques TM, Ortner M, Santana I, Steinacker P, Verbeek MM, Volk AE, Ludolph AC, Otto M. (2022). Serum GFAP differentiates Alzheimer's disease from frontotemporal dementia and predicts MCI-to-dementia conversion. J Neurol Neurosurg Psychiatry. 2022 Apr 27:jnnp-2021-328547.

[19] Rademacher TD, Meuth SG, Wiendl H, Johnen A, Landmeyer NC. (2023). Molecular biomarkers and cognitive impairment in multiple sclerosis: State of the field, limitations, and future direction - A systematic review and meta-analysis. Neurosci Biobehav Rev. 2023 Mar;146:105035.

[20] Tahami M AA, Byrnes MJ, White LA, Zhang Q. (2022). Alzheimer's Disease: Epidemiology and Clinical Progression. Neurol Ther. 2022 Jun;11(2):553-569.

[21] Virgilio E, Vecchio D, Crespi I, Puricelli C, Barbero P, Galli G, Cantello R, Dianzani U, Comi C. (2022). Cerebrospinal fluid biomarkers and cognitive functions at multiple sclerosis diagnosis. J Neurol. 2022 Jun;269(6):3249-3257.

[22] Wang KK, Yang Z, Zhu T, Shi Y, Rubenstein R, Tyndall JA, Manley GT. (2018). An update on diagnostic and prognostic biomarkers for traumatic brain injury. Expert Rev Mol Diagn. 2018 Feb;18(2):165-180.

[23] Xiang Y, Xin J, Le W, Yang Y. (2020). Neurogranin: A Potential Biomarker of Neurological and Mental Diseases. Front Aging Neurosci. 2020 Oct 6;12:584743.

[24] Xue M, Sun FR, Ou YN, Shen XN, Li HQ, Huang YY, Dong Q, Tan L, Yu JT. (2020). Alzheimer's Disease Neuroimaging Initiative. Association of cerebrospinal fluid neurogranin levels with cognition and neurodegeneration in Alzheimer's disease. Aging (Albany, NY). 2020 May 18;12(10):9365-9379.

[25] Yang J, Korley FK, Dai M, Everett AD. (2015). Serum neurogranin measurement as a biomarker of acute traumatic brain injury. Clin Biochem. 2015 Sep;48(13-14):843-8.