Int Clin Neurosci J. 2023;10:e5 Original Article

doi:10.34172/icnj.2023.05

Serum Matrix Metalloproteinase-9 Level and Previous Disease Activity in Relapsing-Remitting Multiple Sclerosis

Eman Hamdy[1*], Ismail Ramdan[1], Jaidaa Mekky[1], Aya Abdel Gleel[2], Dina Gaber[1]

[1]Department of Neurology, Faculty of Medicine, Alexandria University, Egypt
[2]Departemnt of Radiology, Faculty of Medicine, Alexandria University, Egypt

Abstract

Background: Matrix metalloproteinase-9 (MMP-9) is a marker of blood-brain barrier destruction, that is elevated during clinical relapses in multiple sclerosis (MS). In between relapses, MMP-9 levels decline but remain higher than the normal population. This study aimed to investigate the relation between serum MMP-9 level and disease activity in MS during relapse-free periods.

Methods: This was a retrospective study conducted on adult patients with relapsing-remitting MS (RRMS) whose last relapse was ≥ 1 month ago. Serum MMP-9 was withdrawn at the time of recruitment and correlated with parameters of disease activity.

Results: Of the 40 patients recruited, 75% were women. The mean age was 36.2 ± 8.4 years, and the mean disease duration was 7 years. Patients' median Expanded Disability Status Scale (EDSS) was 3.5 (IQR: 2.5-5.25), the median duration since the last relapse was 3 months, and the median duration since last corticosteroid administration was 6 months. On multivariate regression analysis, there was a significant association between serum MMP-9 levels and duration since the last relapse (B: -0.004, 95% CI: -0.007- -0.002, $P=0.001$) as well as duration since the last corticosteroid intake (B: -0.003, 95% CI: -0.006- -0.001, $P=0.005$).

Conclusion: Serum MMP-9 levels correlated with the duration since last relapse and duration since last corticosteroids administration during relapse-free periods.

Keywords: Disease activity, Multiple sclerosis, Serum matrix metalloproteinase 9, Relapse

Received: June 10, 2022 **Accepted:** October 21, 2022 **Published online:** March 5, 2023

Citation: Hamdy E, Ramdan I, Mekky J, Abdel Gleel A, Gaber D. Serum matrix metalloproteinase-9 level and previous disease activity in relapsing-remitting multiple sclerosis. Clin Neurosci J. 2023;10:e5. doi:10.34172/icnj.2023.05.

Introduction

Maintaining or switching between disease-modifying therapies (DMTs) in relapsing-remitting multiple sclerosis (RRMS) is based largely on breakthrough disease activity and drug tolerability.[1-3] To date, breakthrough disease activity is evaluated via assessment of clinical relapses and evidence of disease activity on follow-up magnetic resonance imaging (MRI).[4] In a non-negligible proportion of patients, confirmation of the presence of clinical relapses remains challenging, and differentiation between true and pseudo-relapses may not always be straightforward.[5] Radiological evidence of activity i.e., detection of gadolinium enhancing T1 lesions remains the gold standard of confirmation of relapses in multiple sclerosis (MS).[6] However, the high cost and hazards associated with frequent gadolinium MRI acquisition make it a less practical tool for activity authentication.[7]

Identification of a reliable peripheral biomarker of breakthrough disease activity is a point of research interest that would help neurologists and MS specialists make accurate decisions during initiating and changing DMTs for their patients. Though several laboratory biomarkers of disease activity have been proposed, a reliable laboratory biomarker remains elusive.[6,8,9]

Matrix metalloproteinase-9 (MMP-9) is a biomarker of blood-brain barrier destruction.[10] It is produced in the epithelial cells of the meninges, and is expressed in microglia, astrocytes, vascular endothelial cells, and accumulated inflammatory cells such as T lymphocytes and monocytes.[11] MMP-9 is involved in proteolysis of the blood-brain barrier and facilitation of leucocytic infiltration into the central nervous system.[11] In animal research, MMP-9 is upgraded in the experimental autoimmune encephalomyelitis, a mouse model of MS.[12] In humans, serum levels of MMP-9 were found to be elevated during clinical relapses of MS and decline after relapse resolution but remain higher than the normal population.[13-15]

As serum MMP-9 level declines gradually after relapses in RRMS,[13-15] it might be an indicator, not only for current relapses but also to previous relapses. To date, the data in the literature are lacking about the presence of a lab marker for the past year level of activity, and only radiological activity on MRI of the brain is used to determine the past year disease activity.[7] In this research, we hypothesized that the serum MMP-9 level could be a marker of such activity, and we aimed to investigate whether serum MMP-9 level was correlated with previous disease activity

***Correspondence to** Eman Hamdy, Email: eman.ameen@Alexmed.edu.eg

during relapse-free periods.

Patients and Methods

Study Design and Patient Selection

This was a retrospective study conducted on 40 adult patients with RRMS during the period between April and June 2021 in the MS unit in Alexandria University Hospital in Egypt. The MS unit was established in 2014 and has approximately 1700 patients registered. Inclusion criteria were adult patients ≥ 18 years old, RRMS diagnosed based on the revised 2017 McDonald's criteria,[16] the last relapse ≥ 1 month prior to recruitment, having a brain MRI one year prior to recruitment, and taking the same disease modifying therapy (i.e., interferon beta1 subcutaneous injections). Patients with comorbid disorders that could affect MMP9 levels (such as stroke, coronary heart disease, arthritis, chronic obstructive pulmonary disease, and metabolic syndrome) were excluded from the study.[17,18] Only those who accepted to participate in the study were included.

Data Collection

Demographic data including age and sex were recorded. Clinical data included disease duration in years, affected functional domains, current EDDS, the total number of relapses, duration since the last relapse (in months), and duration since the last corticosteroids administration (in months).

Disease Activity Assessment

Disease activity variables were categorized into clinical and radiological variables. Clinical variables included the presence of clinical relapses during the past 12 months [i.e., a dichotomous yes/no variable] and the number of relapses during the past 12 months. A follow-up brain MRI using Philips's machine was performed on all patients at the time of recruitment. Imaging was compared with the previously available imaging [i.e., one year ago] for evaluating radiological activity. Radiological activity was defined as the presence of one or more new or enlarging T2 lesions. Radiological activity variables included the presence of disease activity findings (a dichotomous yes/no variable) and the number of new\enlarging T2 lesions during the past 12 months.

Serum Matrix Metalloproteinase 9 Level Measurement

Serum MMP-9 concentrations were evaluated for all recruited patients via a human MMP-9 enzyme-linked immunoassay (ELISA) kit (size 96T) manufactured by Cloude-Clone Corp. (cat no: E-01013) at the time of recruitment.[19,20] The collected serum was separated, allowed to clot for 30 minutes, centrifuged at 3000 × g, and then analyzed using Sandwich-ELISA technique.[21] Standards and samples were added to all Micro-ELISA strip plates, and Horseradish peroxidase (HRP)-conjugate reagent was added to all wells. After an hour of incubation, successive well washing was conducted, and chromogen solutions A and B were added. A second washing was performed, and the 'stop' solution was added until color change to yellow occurred. To quantify the color intensity, a spectrometer was used, and the color was measured at 450 nm. A standard curve of the optical density was produced from the samples and the calibration standards (Figure 1) to calculate the concentrations of the MMP-9 levels. The sensitivity of this assay was estimated to be 0.1 ng/mL.[20]

Statistics and Data Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of the distribution of variables. Mean and standard deviation were used to summarize parametric quantitative variables. Median and 25th to 75th interquartile range (IQR) were used to summarize non-parametric quantitative variables. Categorial variables were summarized as numbers and percentages. Mann–Whitney U test was used to compare the median levels of serum MMP-9 among patients with clinical relapses during the past 12 months and those without clinical relapses, and to compare serum MMP-9 levels among patients with and without radiological progression. Spearman coefficient was used to test the correlation between serum MMP-9 levels and the duration since last relapse, duration since last corticosteroid intake, number of relapses in the past 12 months, and number of new/enlarging T2 MRI lesions. Linear regression analysis was used to detect the most independent/affecting factor influencing MMP9. The significance of the obtained results was judged at the 5% level.

Results

Forty patients with RRMS were recruited to this study. Of the studied sample, 75% (n = 30) were women. The mean age of patients was 36.2 ± 8.4 years, and the median disease duration was 7 (IQR: 6-8) years. The patients' EDSS ranged from 1.0 to 6.0 with a median of 3.5 (IQR:

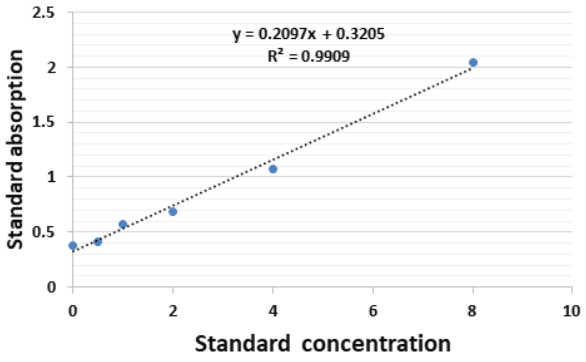

Figure 1. The Standard Curve Generated From the Patients and Controls.

2.5-5.25). Table 1 details the demographic, clinical, and radiological characteristics of the recruited patients. The median number of total relapses the patients experienced in their life was 5 (IQR: 4-7), and the median duration since the last relapse was 3 (IQR: 1-10.5) months. As not all relapses required corticosteroid treatment, the duration since the last corticosteroids intake was longer i.e., 6 (range: 2-32) months (Table 1).

Twenty-seven (67.5%) patients reported they had one or more relapses during the previous 12 months, and the median number of relapses was 1 (IQR: 0-2) relapse. As for the radiological activity, 29 patients (72.5%) had evidence of progression on follow-up brain MRI. The median number of new/enlarging T2 lesions was 5 (IQR: 0-7) lesions (Table 1). As all patients recruited had no relapses during the past month, none of them had gadolinium-enhancing lesions on brain MRI.

The median level of serum MMP-9 among the recruited patients was 1.24 (IQR 0.93-1.43) ng/mL. On comparing serum MMP-9 among patients according to their previous year activity, no significant difference was found in the serum MMP-9 between patients with and without evidence of radiological activity during the past 12 months ($P=0.301$). Serum MMP-9 levels were significantly correlated with the number of relapses during the past 12 months ($r=0.206$, $P<0.001$), the number of new/enlarging T2 brain lesions on follow-up brain MRI ($r=0.113$, $P=0.011$), and duration since last relapse ($r=-0.316$, $P<0.001$), and the duration since the last corticosteroid intake ($r=-0.323$, $P<0.001$) (Table 2).

Multivariate linear regression analysis was performed for the parameters that might affect serum MMP9 levels (Table 3). The regression analysis showed that the duration since the last relapse and the duration since the last corticosteroids administration were significantly correlated with the serum MMP-9 levels.

Discussion

Determination of annual MS disease activity is fundamental during the journey of MS treatment.[2] Clinical confirmation of relapses is often challenging, partly due to the inappropriate interpretation of pseudo-relapses and partly due to subclinical disease activity that is missed by both patients and physicians.[4] MRI activity has long been considered the best surrogate marker of MS activity.[22] However, its high cost, non-availability during relapses, and gadolinium-related complications reduce its applicability.[7] Therefore, a peripheral lab marker for the previous disease activity would be of help. To date, however, no peripheral lab marker has been identified to correlate with the past year disease activity. In this research, a hypothesis that serum MMP-9 could be a biomarker for MS previous activity was tested. The hypothesis was made based on the data from literature about the role of MMP-9 in the disruption of BBB and

Table 1. Demographic and Clinical Characteristics of Recruited Patients (n = 40)

Demographic and Clinical Characteristics	Description
Age[a]	36.2 ± 8.4 years
MS disease duration[b]	7 (6-8) years
Total number of relapses[b]	5 (4-7) relapse
Duration since last relapse[b]	3 (1-10.5) months
Duration since last corticosteroids intake[b]	6 (2-32) months
Number of relapses in the previous 12 months	1 (0-2) relapses
Number of new/enlarging T2 lesions during the previous 12 months[b]	5 (0-7)
Serum MMP9 levels[b]	1.24 (0.93-1.43) ng/mL
EDSS[b]	3.5 (2.5-5.25)
	Number of Patients (%)
Gender	
Male	10 (25%)
Female	30 (75%)
Functional domains affected	
Visual	27 (72.5%)
Brainstem	16 (40%)
Pyramidal	35 (87.5%)
Cerebellar	35 (87.55)
Sensory	40 (100%)
Bowel/bladder	28 (70%)
Ambulation	32 (80%)
Patients who experienced ≥ 1 relapses during the previous 12 months	27 (67.5%)
Last MRI findings:	
Periventricular lesions	40 (100%)
< 9 lesions	29 (72.5%)
≥ 9 lesions	11 (27.5%)
Cortical/juxtacortical lesions	17 (42.5%)
Infratentorial lesions	32 (80%)
T1 black holes	26 (65%)
Radiological activity during the previous 12 months (yes/no)	29 (72.5%)

Abbreviations: EDSS, Expanded disability status scale; MMP9, matrix metalloproteinase 9; MRI, magnetic resonance imaging; MS, multiple sclerosis; SD, standard deviation.
[a] Mean ± standard deviation, [b] Median and interquartile range.

Table 2. Relation Between the Serum MMP9 Levels and Parameters of Disease Activity (n = 40)

	Serum MMP-9 Level (g/L)	*P* Value
Clinical relapses		
Yes	1.30 (0.97-1.68)	< 0.001*
No	1.14 (1.12-1.36)	
Radiological progression		
Yes	1.28 (0.97-1.65)	0.301
No	1.33 (1.12-1.36)	
Duration since last relapse	-0.316	< 0.001*
Duration since last corticosteroid intake	-0.323	< 0.001*
Number of relapses in the past 12 months	0.206	< 0.001*
Number of new/enlarging T2 MRI lesions	0.113	0.011*

Abbreviations: MMP9, matrix metalloproteinase 9; SD, standard deviation.
*P value < 0.05.

Table 3. Linear Regression Analysis for the Parameters Affecting Serum MMP9 Levels (n=40)

	B	95% CI	*P*	B	95% CI	*P*
Clinical relapses	0.217	0.136-0.298	<0.001*	0.133	-0.19- -0.001	0.087
MRI activity	0.038	-0.051- -0.127	0.401			
Duration since last CST intake	-0.006	-0.007- -0.004	<0.001*	-0.003	-0.006- -0.001	0.005*
Duration since last relapse	-0.007	-0.009- -0.005	<0.001*	-0.004	-0.007- -0.002	0.001*
Past year number of relapses	0.090	0.053-0.128	<0.001*	-0.052	-0.019- 0.284	0.349
Number of new lesions in MRI	0.008	0.002-0.015	<0.001*	0.006	-0.006- 0.018	0.301

Abbreviations: B, unstandardized coefficient; CI, confidence interval; CST, corticosteroids; MRI, magnetic resonance imaging.
**P* value<0.05.

induction of inflammation.[11] Even in-between relapses, patients with RRMS continued to have elevated serum MMP-9 levels in comparison to their counterparts from a healthy population.[23] To test this proposed hypothesis, we recruited patients with RRMS who had been free of relapses for at least one month and measured their serum MMP-9 level and correlated it with the previous clinical ad radiological disease activity.

During patient selection, a homogenous group of patients was targeted. All patients had been treated with the same types of DMTs (i.e., interferon beta-1a) for approximately 2 years. To eliminate the effect of relapse on serum MMP-9 level, all patients were recruited after at least one month of relapses. Serum MMP-9 levels were significantly higher among patients who had clinical relapses during the past 12 months. Moreover, the serum levels were negatively correlated with the duration since last relapse and the duration since last corticosteroids intake. As aforementioned, many patients report pseudo-relapses as disease activity and others cannot differentiate between a new relapse and a residual from previous relapses. Many patients also develop subclinical relapses. Therefore, a biomarker that correlates better with previous activity is more reliable, as is the case in this research. The findings from this research support the hypothesis that serum MMP-9 can be a potential biomarker of previous activity.

In the literature, scarce data exists about the presence of a peripheral laboratory marker for previous year activity. Markers such as neurofilaments light chain, neurofilaments heavy chain, glial fibrillary acidic protein, chitinase-3-like-1 (CHI3L1), cytokines (e.g., CXCL1, CXCL8, CXCL10, CXCL13, CCL22, and HMGB1), and osteopontin have been tested as biomarkers of disease activity, but no sufficient evidence exists for their reliability.[24-28] For the MMP-9, patients with MS had higher serum and cerebrospinal fluid (CSF) levels of MMP-9 compared with their counterparts from the general population.[29-32] The levels during relapses were significantly higher than in-between relapses making it a potential marker of current disease activity.[23,29,32-36] In the literature, all the studies were concerned with measuring serum MMP-9 during relapses,[23,29,32-36] and few of them monitored serum levels in-between relapses and found that they decline following relapse remission but remain higher than the healthy controls.[13-15] In this research, we did not aim to assess the MMP-9 as a marker for current disease activity, as performed in most studies, but we investigated its correlation with the presence or absence of clinical and/or radiological activity which makes our findings novel.

The strength of this research is that it is, to the best of our knowledge, the first to evaluate serum MMP-9 as a potential biomarker of previous MS disease activity. The main limitations are the relatively small sample size and measuring MMP-9 at one point of time. Future research is needed to obtain data (serum MMP9 level, MRI) at several different time points to confirm their association.

Conclusion

Serum MMP-9 level can be a potential marker of previous disease activity. Serum MMP-9 levels were found to be correlated with the disease activity, the duration since last relapse and duration since last corticosteroids administration during relapse-free periods.

Competing Interests

The authors have no conflicts of interest to declare.

Ethical Approval

Ethical approval was obtained from the Ethics Committee (EC) of Alexandria University Faculty of Medicine (IRB number 00012098) which operates according to the International Conference of Harmonization Good Clinical Practice (ICH GCP) and applicable local and institutional regulations and guidelines[37]. The EC has a federal-wide assurance (FWA)[38] from 2010 (FWA number 00018699).

Funding

No funds were received for this research.

References

1. Bigaut K, Cohen M, Durand-Dubief F, Maillart E, Planque E, Zephir H, et al. How to switch disease-modifying treatments in multiple sclerosis: guidelines from the French Multiple Sclerosis Society (SFSEP). Mult Scler Relat Disord. 2021;53:103076. doi: 10.1016/j.msard.2021.103076.
2. Miller AE. Switching or discontinuing disease-modifying therapies for multiple sclerosis. Continuum (Minneap Minn).

2016;22(3):851-63. doi: 10.1212/con.0000000000000327 .
3. Corboy JR, Weinshenker BG, Wingerchuk DM. Comment on 2018 American Academy of Neurology guidelines on disease-modifying therapies in MS. Neurology. 2018;90(24):1106-12. doi: 10.1212/wnl.0000000000005574.
4. Fox EJ, Havrdova E. Breakthrough disease in multiple sclerosis–the problem and treatment options. Eur Neurol Rev. 2013;7(Suppl 2):24-31. doi: 10.17925/enr.2013.08.s1.24 .
5. Merwick A, Sweeney BJ. Functional symptoms in clinically definite MS--pseudo-relapse syndrome. Int MS J. 2008;15(2):47-51.
6. Mills EA, Mirza A, Mao-Draayer Y. Emerging approaches for validating and managing multiple sclerosis relapse. Front Neurol. 2017;8:116. doi: 10.3389/fneur.2017.00116 .
7. Radbruch A. Are some agents less likely to deposit gadolinium in the brain? Magn Reson Imaging. 2016;34(10):1351-4. doi: 10.1016/j.mri.2016.09.001.
8. Graber JJ, Dhib-Jalbut S. Biomarkers of disease activity in multiple sclerosis. J Neurol Sci. 2011;305(1-2):1-10. doi: 10.1016/j.jns.2011.03.026.
9. Huang J, Khademi M, Fugger L, Lindhe Ö, Novakova L, Axelsson M, et al. Inflammation-related plasma and CSF biomarkers for multiple sclerosis. Proc Natl Acad Sci U S A. 2020;117(23):12952-60. doi: 10.1073/pnas.1912839117.
10. Rempe RG, Hartz AMS, Bauer B. Matrix metalloproteinases in the brain and blood-brain barrier: versatile breakers and makers. J Cereb Blood Flow Metab. 2016;36(9):1481-507. doi: 10.1177/0271678x16655551.
11. Mirshafiey A, Asghari B, Ghalamfarsa G, Jadidi-Niaragh F, Azizi G. The significance of matrix metalloproteinases in the immunopathogenesis and treatment of multiple sclerosis. Sultan Qaboos Univ Med J. 2014;14(1):e13-25. doi: 10.12816/0003332.
12. Toft-Hansen H, Nuttall RK, Edwards DR, Owens T. Key metalloproteinases are expressed by specific cell types in experimental autoimmune encephalomyelitis. J Immunol. 2004;173(8):5209-18. doi: 10.4049/jimmunol.173.8.5209.
13. Trentini A, Castellazzi M, Cervellati C, Manfrinato MC, Tamborino C, Hanau S, et al. Interplay between matrix metalloproteinase-9, matrix metalloproteinase-2, and interleukins in multiple sclerosis patients. Dis Markers. 2016;2016:3672353. doi: 10.1155/2016/3672353.
14. Voloshyna N, Vasylovskyy V, Nehreba T, Chernenko M, Vovk V. Matrix metalloproteinase-9 and inflammation in different types of multiple sclerosis. EUREKA: Health Sciences. 2016;1:39-44. doi: 10.21303/2504-5679.2016.00039.
15. Benešová Y, Vašků A, Novotná H, Litzman J, Štourač P, Beránek M, et al. Matrix metalloproteinase-9 and matrix metalloproteinase-2 as biomarkers of various courses in multiple sclerosis. Mult Scler. 2009;15(3):316-22. doi: 10.1177/1352458508099482.
16. Aktas O, Wattjes MP, Stangel M, Hartung HP. [Diagnosis of multiple sclerosis: revision of the McDonald criteria 2017]. Nervenarzt. 2018;89(12):1344-54. doi: 10.1007/s00115-018-0550-0. [German].
17. Snitker S, Xie K, Ryan KA, Yu D, Shuldiner AR, Mitchell BD, et al. Correlation of circulating MMP-9 with white blood cell count in humans: effect of smoking. PLoS One. 2013;8(6):e66277. doi: 10.1371/journal.pone.0066277.
18. Kieseier BC, Kiefer R, Clements JM, Miller K, Wells GM, Schweitzer T, et al. Matrix metalloproteinase-9 and -7 are regulated in experimental autoimmune encephalomyelitis. Brain. 1998;121(Pt 1):159-66. doi: 10.1093/brain/121.1.159.
19. CLOUD-CLONE CORP (CCC). ELISA Kit for Matrix Metalloproteinase 9 (MMP9) | SEA553Hu | Homo sapiens (Human) CLOUD-CLONE CORP (CCC). http://www.cloud-clone.com/products/SEA553Hu.html. Accessed October 1, 2021.
20. BioVendor. Human Matrix Metalloproteinase-9 Elisa. https://www.biovendor.com/file/6762/PDS_HuMMP9_ELISA_ENG_001.pdf. Accessed August 6, 2021.
21. Konstantinou GN. Enzyme-linked immunosorbent assay (ELISA). In: Lin J, Alcocer M, eds. Food Allergens: Methods and Protocols. Vol 1592. New York, NY: Springer; 2017. p. 79-94. doi: 10.1007/978-1-4939-6925-8_7.
22. Sormani MP, De Stefano N. Defining and scoring response to IFN-β in multiple sclerosis. Nat Rev Neurol. 2013;9(9):504-12. doi: 10.1038/nrneurol.2013.146.
23. Matrix metalloproteinase 9 as a marker of disease activity in multiple sclerosis. Nat Clin Pract Neurol. 2006;2(9):464. doi: 10.1038/ncpneuro0231 .
24. Harris VK, Tuddenham JF, Sadiq SA. Biomarkers of multiple sclerosis: current findings. Degener Neurol Neuromuscul Dis. 2017;7:19-29. doi: 10.2147/dnnd.s98936.
25. Bucova M, Majernikova B, Durmanova V, Cudrakova D, Gmitterova K, Lisa I, et al. HMGB1 as a potential new marker of disease activity in patients with multiple sclerosis. Neurol Sci. 2020;41(3):599-604. doi: 10.1007/s10072-019-04136-3.
26. Håkansson I. Biomarkers and Disease Activity in Multiple Sclerosis: A Cohort Study on Patients with Clinically Isolated Syndrome and Relapsing Remitting Multiple Sclerosis. Linköping University Electronic Press; 2019.
27. Bittner S, Oh J, Havrdová EK, Tintoré M, Zipp F. The potential of serum neurofilament as biomarker for multiple sclerosis. Brain. 2021;144(10):2954-63. doi: 10.1093/brain/awab241.
28. Novakova L, Zetterberg H, Sundström P, Axelsson M, Khademi M, Gunnarsson M, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology. 2017;89(22):2230-7. doi: 10.1212/wnl.0000000000004683.
29. Latronico T, Liuzzi GM. Metalloproteinases and their inhibitors as therapeutic targets for multiple sclerosis: current evidence and future perspectives. Metalloproteinases Med. 2017;4:1-13. doi: 10.2147/mnm.s88655.
30. Castellazzi M, Ligi D, Contaldi E, Quartana D, Fonderico M, Borgatti L, et al. Multiplex matrix metalloproteinases analysis in the cerebrospinal fluid reveals potential specific patterns in multiple sclerosis patients. Front Neurol. 2018;9:1080. doi: 10.3389/fneur.2018.01080.
31. Amini R, Karampoor S, Zahednasab H, Keyvani H, Gheiasian M, Azizi Jalilian F. Serum levels of matrix metalloproteinase-2, -9, and vitamin D in patients with multiple sclerosis with or without herpesvirus-6 seropositivity. Braz J Infect Dis. 2020;24(2):144-9. doi: 10.1016/j.bjid.2020.02.001.
32. Fainardi E, Castellazzi M, Bellini T, Manfrinato MC, Baldi E, Casetta I, et al. Cerebrospinal fluid and serum levels and intrathecal production of active matrix metalloproteinase-9 (MMP-9) as markers of disease activity in patients with multiple sclerosis. Mult Scler. 2006;12(3):294-301. doi: 10.1191/135248506ms1274oa.
33. Yılmaz U, Gücüyener K, Gürkaş E, Demir E, Serdaroğlu A, Atak A, et al. Matrix metalloproteinase-7, matrix metalloproteinase-9, and disease activity in pediatric multiple sclerosis. Pediatr Neurol. 2013;48(3):255-6. doi: 10.1016/j.pediatrneurol.2012.12.003.

34. Trentini A, Manfrinato MC, Castellazzi M, Tamborino C, Roversi G, Volta CA, et al. TIMP-1 resistant matrix metalloproteinase-9 is the predominant serum active isoform associated with MRI activity in patients with multiple sclerosis. Mult Scler. 2015;21(9):1121-30. doi: 10.1177/1352458514560925.
35. Romi F, Helgeland G, Gilhus NE. Serum levels of matrix metalloproteinases: implications in clinical neurology. Eur Neurol. 2012;67(2):121-8. doi: 10.1159/000334862.
36. Avolio C, Ruggieri M, Giuliani F, Liuzzi GM, Leante R, Riccio P, et al. Serum MMP-2 and MMP-9 are elevated in different multiple sclerosis subtypes. J Neuroimmunol. 2003;136(1-2):46-53. doi: 10.1016/s0165-5728(03)00006-7.
37. ICH GCP. ICH GCP (Good Clinical Practice) Training Course. https://ichgcp.net/. Published 2011. Accessed April 4, 2020.
38. HHS. Federalwide Assurance (FWA) for the Protection of Human Subjects | HHS.gov. https://www.hhs.gov/ohrp/register-irbs-and-obtain-fwas/fwas/fwa-protection-of-human-subjecct/index.html. Accessed August 6, 2021.