Tabari Biomedical Student Research Journal

OPEN ACCESS

ORIGINAL: Investigating the Role of Osteopontin as a Potential Biomarker in Relapsing-Remitting Multiple Sclerosis

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ARTICLE INFO

Submitted: 11 Apr 2025
Accepted: 13 May2025
Published: 24 Jun 2025

Keywords:
Cytomegalovirus
Infection
Hemodialysis
IgM
IgG

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Citation:
Frouzanian M, Ashoori A, Baghbanian M, Ghasemi Hamidabadi H. Investigating the Role of Osteopontin as a Potential Biomarker in Relapsing-Remitting Multiple Sclerosis. Tabari Biomed Stu Res J. 2025;7(3): 30- 35.

ABSTRACT

Introduction: Multiple sclerosis is a chronic central nervous system disorder with an unknown etiology. Osteopontin is a pro-inflammatory cytokine that has been implicated in MS pathogenesis. This study aimed to investigate OPN levels in relapsing-remitting MS patients and compare them with healthy individuals to determine its potential role as a biomarker.

Methods: A case-control study was conducted on MS patients referred to BooAli Hospital in Sari. Fifty relapsing-remitting MS patients and 30 healthy controls were included. Plasma OPN levels were assessed using ELISA kits, and we considered the p-value < 0.05 significant.

Findings: Among the participants, the MS group was composed of 30 women (60%) and 20 men (40%), while the healthy group comprised 20 women (66.7%) and ten men (33.3%). Notably, OPN levels were significantly elevated in the MS group compared to the healthy individuals (p-value < 0.001). No difference in OPN levels was observed between male and female MS patients (p-value = 0.4). A significant inverse correlation was also identified between the EDSS score and OPN levels (p-value = 0.01).

Conclusion: This study demonstrates elevated OPN levels in relapsing-remitting MS patients compared to healthy individuals, suggesting its potential as a biomarker for disease activity in MS.

Introduction

Multiple sclerosis (MS) is an enduring and progressive ailment affecting the central nervous system, characterized by inflammation, demyelination, damage to axons, and subsequent neurodegeneration [1]. The global incidence of MS has increased since 2013, with an estimated 2.8 million individuals, corresponding to a rate of 35.9 per 100,000 people, believed to be living with this condition worldwide [2]. Globally, it is among the leading factors that result in disability among young adults. Notably, women face a greater susceptibility to developing MS when compared to men [3, 4]. While its exact origins remain partially shrouded in mystery, MS is thought to involve immune mechanisms that target myelin antigens. Both genetic factors and external triggers, such as viral infections, metabolic

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influences, and environmental factors, contribute to the development of this autoimmune disorder. MS manifests in unpredictable symptoms, often commencing with episodes of temporary neurological impairments and progressing toward permanent disability in the form of relapsing-remitting disease. Comprehensive diagnostic measures encompass a thorough medical history evaluation, clinical examination, and imaging techniques like MRI.

Osteopontin (OPN) is an extracellular matrix protein involved in various physiological functions and pathological states. It has been implicated in bone regeneration, wound healing, cancer biology, vascular disorders, and inflammatory diseases [5, 6]. OPN is expressed in immune cells and plays a role in inflammation by influencing the production of cytokines and inhibiting anti-inflammatory factors [7, 8]. Given its potential role in autoimmune disorders, including MS, the relationship between OPN and MS has been extensively studied (9-12). Various studies have shown that OPN gene variants affect the risk of developing MS and disease progression [13, 14]. Higher OPN levels have been associated with disease progression and frequent relapses, suggesting its potential as a biomarker for MS [15, 16].

OPN has been shown to modulate interleukin synthesis and increase the levels of pro-inflammatory cytokines in T lymphocytes. A previous study in mice demonstrated that OPN deficiency reduces the severity of MS and improves recovery, while increased OPN expression has been observed in active MS lesions [17]. OPN is also involved in secondary neurodegeneration and is found at higher levels in the cerebrospinal fluid and plasma of MS patients [9].

Researchers have sought to identify reliable biomarkers for detecting MS, and one such candidate is OPN. It plays a crucial role in immune response regulation and the development of immune-mediated and inflammatory diseases. OPN is linked to pathogenic T cells, particularly T helper 17 cells, where it supports IL-17 expression and contributes to pathology [18]. OPN stimulation enhances the expression of CD40 ligand (CD40L) and IFN-gamma on human T cells, producing IL-12. This suggests that OPN regulates T cell-dependent IL-12 production, which is necessary for initiating a protective response against intracellular pathogens [19]. Many studies indicated that OPN is closely associated with MS and plays a significant role in the disease course [20-22].

Although the current treatment strategy for MS primarily focuses on reducing the frequency of attacks, halting disease progression, and minimizing resultant disabilities, investigations into the role of OPN in MS pathogenesis continue to offer insights for potential therapeutic interventions [23]. Therefore, this study aimed to determine the level of OPN in relapsing MS patients undergoing recovery and to compare it with healthy individuals.

Methods

This case-control study was conducted on individuals diagnosed with multiple sclerosis and referred to Bo Ali Hospital in Sari. A total of 50 patients with relapsing-remitting type multiple sclerosis was included in the study, diagnosed by neurologists from BooAli Hospital in Iran. The diagnosis was based on McDonald's criteria, which considered Evoked Potential (EP), Magnetic Resonance Image (MRI), and Cerebrospinal Fluid (CSP) findings. To form the control group, 30 healthy individuals without a history of autoimmune diseases were randomly selected and matched with the patient group in terms of gender and age.

Participants meeting the study's inclusion criteria were enrolled, which included a disease duration of at least five years and disease onset at the age of 25 years. However, individuals with the following exclusion criteria were not included: those with organic failures such as heart failure, liver and kidney failure, lung failure, depression, or epilepsy; pregnant or lactating patients; individuals with other types of multiple sclerosis; and those who had received prior treatment with corticosteroid drugs before entering the study.

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These criteria were established to ensure that the study focused specifically on individuals with specific disease duration and age of onset while excluding individuals with comorbidities or confounding factors that could impact the results.

Procedures

Following an overnight fasting period, venous blood samples were obtained from all participants. These samples were then transferred to sterile 10 ml tubes. After centrifugation, the plasma was separated and stored at -80°C for subsequent analysis. The plasma concentration of OPN was measured using enzyme-linked immunosorbent assay (ELISA) kits. The Extended Disability Status Scale (EDSS) was employed to assess the disability level of MS patients included in the study. The EDSS scale ranges from 0 (indicating a routine neurological examination) to 10 (representing death due to MS). Based on the EDSS scores, the patients were categorized into three groups: mild (0-3), moderate (4-6), and severe (7) or higher.

Ethical Considerations

The researchers upheld the confidentiality of information and anonymity of study participants following the Helsinki Declaration. Before the study, the research procedures and the participants' involvement were thoroughly explained to each patient individually. Before enrolling in the study, each participant provided written informed consent.

Statistical Analysis

We assessed the normal distribution of plasma OPN levels using the Kolmogorov-Smirnov Z test. As appropriate, we performed statistical analyses using the Mann-Whitney U, Kruskal-Wallis, and Pearson Chi-Squared tests. We reported the results as mean ± standard deviation and considered a significance p-value less than 0.05 statistically significant.

Results

During the designated investigation period, a cohort of 50 patients afflicted with relapsing-remitting multiple sclerosis (RRMS), as determined by McDonald's criteria, were duly identified and incorporated into the study. The control group, serving as a comparative reference, comprised 30 individuals in good health. Among the RRMS group, there were 30 women (60%) and 20 men (40%), while the healthy group consisted of 20 women (66.7%) and ten men (33.3%). The mean age within the RRMS group was 34.5 ± 11.1 years, while the healthy group exhibited a mean age of 33.2 ± 11.5 years. Statistical analysis revealed no significant disparity in gender distribution (p = 0.68) or mean age (p = 0.9) between the two cohorts. Notably, the average duration of MS diagnosis among the patients amounted to 3 ± 1.1 years and a minority of 5 individuals (10%) presented with a familial history of MS.

The application of ANOVA analysis revealed a significant distinction in the concentration of OPN between the MS group and the control group, indicating a statistically significant difference (Table 1 and 2).

Table 1. Comparison of average OPN levels between multiple sclerosis and control groups

Group		OPN level (mean ± SD)	P-value
Sex	**Male**	11.13 ± 4.9	0.4
	Female	11.8 ± 4.3	
Control		6.87 ± 0.4	<0.001

Table 2. The relationship between EDSS and serum OPN levels in patients with multiple sclerosis

EDSS	Frequency	OPN levels	P-value
0 - 3	22	11.9 ± 3.6	0.01
3.5 - 7	28	8.6 ± 2.5	
7.5 - 10	0	0	

EDSS; The Extended Disability Status Scale, OPN; Osteopontin

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Discussion

OPN has been implicated in the pathogenesis of MS and has garnered interest as a potential biomarker for disease activity [24]. The present study aimed to investigate OPN levels in MS patients and compare them with healthy individuals to further elucidate its potential role in the disease.

In line with prior research, this study's results indicated a significant increase in OPN levels among MS patients compared to healthy controls [9, 22]. This supports the notion that OPN plays a crucial role in the immunopathology of MS. OPN is known to promote the recruitment and activation of immune cells, contributing to the inflammatory processes observed in MS [19]. Furthermore, OPN has been implicated in developing and progressing MS-related lesions, suggesting its involvement in disease severity. [1, 2, 4].

Jafarinia and colleagues conducted a case-control study comparing plasma OPN levels in relapsing-remitting MS patients during remission with healthy individuals. Their findings revealed significantly higher OPN levels in the RRMS group compared to the control group. The study suggested that the increased OPN levels in RRMS patients during the remission phase may reflect a pro-inflammatory cytokine environment [12]. These results align with our research, further supporting the potential of targeting OPN as a therapeutic strategy for multiple sclerosis.

Regarding gender differences, this study did not find a significant disparity in OPN levels between male and female MS patients. No study has explicitly explored the gender distribution of OPN levels among MS patients. Researchers must address this unexplored area in future investigations, as it represents a knowledge gap that requires further studies.

A noteworthy finding of this study is the inverse relationship observed between OPN levels and the Expanded Disability Status Scale (EDSS) score. The EDSS is a widely used measure of disability in MS patients, with higher scores indicating a more significant disability. The inverse correlation could suggest that higher OPN levels are maybe associated with lower disability scores and show a potential protective effect of OPN against disease progression. Our findings diverge from numerous preceding studies that have consistently implicated elevated levels of OPN in the advancement of MS [20, 22]. Additionally, this finding contrasts with the study by Jafarinia in this aspect, which did not find a significant relationship between plasma OPN levels and the EDSS score in RRMS patients [12]. However, it is essential to acknowledge that our research was constrained by a limited sample size, which may have influenced the observed results. Conducting additional systematic and comprehensive studies to elucidate the genuine relationship between OPN and MS is imperative, ensuring a more conclusive understanding of this complex association.

In a study by Szalardy in 2013, OPN levels were measured in the CSF of MS patients, showing significantly higher levels in the MS group than in the control group [25]. Although our study measured OPN levels in serum rather than CSF, the similarity in findings suggests a consistent association between elevated OPN levels and MS pathology. This highlights the potential of OPN as a biomarker for disease activity and progression in MS and a potential therapeutic target for developing novel treatment strategies.

According to a study conducted by Clemente in 2017, it was found that OPN exhibits significant expression in demyelinating lesions observed in MS and experimental autoimmune encephalomyelitis (EAE), which serves as a mouse model for MS. The study demonstrated that the administration of recombinant OPN leads to relapses, whereas the treatment involving anti-OPN antibodies effectively mitigates the progression of the disease. His study also showed that in the serum of MS patients, levels of anti-OPN autoantibodies were higher in relapsing-remitting MS patients compared to primary- and secondary-progressive MS patients and healthy controls. In a mouse model of EAE, vaccination with OPN-induced antibodies

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against OPN decreased disease severity and correlated with reduced T cell secretion of interleukin 17 [26].

Laffaldano and colleagues conducted a study investigating the effects of two years of Natalizumab treatment on plasma OPN levels, cognitive performance, and fatigue in patients with RRMS. Baseline OPN levels were higher in patients than healthy controls but similar to treatment-naïve RRMS patients. Throughout treatment, there was a significant decrease in OPN levels, improved cognitive function, and reduced fatigue. Baseline OPN levels correlated with cognitive impairment, and the reduction in OPN levels was associated with enhanced cognition during Natalizumab treatment. They concluded that Natalizumab treatment leads to reduced OPN levels, improved cognition, and reduced fatigue in RRMS patients, with cognitive improvement linked to decreased OPN levels [27].This research had a few limitations that should be noticed. Firstly, the sample size used in this research was relatively small, which could limit the generalizability of the findings to a larger population. Additionally, the study was conducted within a single center, which restricts the external validity of the results. Another limitation lies in the study's cross-sectional design, which only allows for assessing associations between variables at a specific moment. Conducting longitudinal studies would be beneficial to explore the temporal relationship between OPN levels and disease activity over an extended duration. Moreover, the lack of long-term follow-up in the study prevented evaluation of the predictive value of OPN levels for disease progression and future relapses. Incorporating long-term data collection would have provided more comprehensive insights into the role of OPN as a biomarker in multiple sclerosis. The findings of this study contribute to the growing body of evidence implicating OPN as a potential biomarker for disease activity in MS. Further research, utilizing larger sample sizes and exploring the underlying mechanisms, is warranted to validate these findings and assess the clinical utility of OPN as a diagnostic or prognostic marker in MS.

Conclusion

Our study demonstrates elevated levels of OPN in relapsing-remitting MS patients compared to healthy individuals. However, the limitations of our study warrant caution in generalizing the findings. Future studies with more extensive and diverse populations are recommended to provide a more comprehensive understanding of the relationship between OPN levels and MS.

Acknowledgment

The authors would like to thank Mazandaran University of Medical Sciences for the support, cooperation and assistance throughout the period study.

Conflicts of interest

The authors declare they have no conflicts of interest and have no financial interest related to any aspect of the study.

Authors' contributions

All authors contributed to the study's design, data analysis, manuscript drafting, and critical revisions, approved the final version for submission, and accepted accountability for the work.

Funding

This study did not receive funding.

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