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Relationship between Osteopontin Blood Marker and Relapse, Remission of Multiple Sclerosis in Saudi Arabia 2024

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ABSTRACT: Background

Osteopontin (OPN) is a widely expressed acidic glycoprotein, and is considered as an interesting biomarker because of its role in the pathophysiology of several inflammatory, degenerative, autoimmune, and oncologic diseases. This study aimed to evaluate Relationship between osteopontin blood marker and relapse, remission of multiple sclerosis in Saudi Arabia patients in 2024 and correlate it with disease activity.

Patients and methods This case–control study recruited consecutively 90 patients divided into two groups: group I includes 30 age-matched and sex-matched healthy individuals as control group, and group II includes 60 RRMS patients, which in turn was subdivided into two subgroups: group IIa including 30 patients in remission and group IIb including 30 patients in relapse before receiving methyl prednisolone. All patients were subjected to full history taking, neurological examination using Expanded Disability Status Scale assessment, and laboratory investigations, including complete blood count, aspartate aminotransferase, alanine aminotransferase, and OPN-level measurement.

Results A highly significant difference between group I and group II as regards OPN level ($P < 0.001$). Receiver operating characteristic curve for OPN level between group I and group II showed that the cutoff level of more than 8 can discriminate between both groups with 88.33% sensitivity and 100% specificity. There was a significant correlation between OPN level and AST ($P < 0.05$).

Conclusion: OPN can be used as an inflammatory biomarker to differentiate between RRMS patients and healthy individuals but cannot discriminate between remission and relapse in MS patients.

INTRODUCTION

Multiple sclerosis (MS) is the most common immune-mediated disorder of the CNS. The disease course is usually characterized by an initial relapsing-remitting phase (RRMS), defined by new neurologic symptoms and subsequent disability (Marastoni et al., 2024; Hemmer et al., 2015; Dendrou et al., 2015). Later in the disease course, most patients develop a progressive accumulation of disability, mostly independent of relapses (secondary progressive MS, SPMS). Over 2.5 million people worldwide are afflicted by multiple sclerosis (MS). The prevalence of MS is increasing in Saudi Arabia. According to the most recent estimation in 2018, the projected prevalence of MS in Saudi Arabia is 40.40/100,000 individuals. This figure places Saudi Arabia above the low-risk zone in terms of MS prevalence (Alnajashi et al., 2024).

MS is a chronic, immune-mediated, demyelinating illness of the central nervous system. The disease usually occurs in young adulthood communities, between 20 and 40 years of age, most commonly in women, and it is the most common cause of disability in young adults after road traffic accidents (Dendrou et al., 2015).MS usually manifests as relapsing-remitting MS (RRMS) or progressive MS, which may be progressive from the time of beginning (primary progressive MS; PPMS) or begin as RRMS and proceed to a progressive disability (secondary progressive MS; SPMS) (Lublin et al., 2014).Disease progression secondary to neurodegeneration and axonal loss is the driving factor for disability and loss of function. There is no cure for MS, but disease-modifying therapies (DMTs) decrease the relapse rate and probably slow or prevent disease progression.

Clinically, MS patients are classified into four major groups: clinically isolated syndrome; an initial clinical presentation of MS, and relapsing–remitting multiple-sclerosis (RRMS); the most common type of MS, and primary progressive MS; clinical progressive disease without recovery, and secondary progressive MS (SPMS); which usually develops after several years of relapsing–remitting disease (Lublin et al., 2014).

MS is characterized by the breakdown of the blood–brain barrier (BBB) at the onset followed by oligodendrocyte loss, demyelination, astrocyte gliosis, and axonal degeneration that resulted in formation of CNS plaques containing inflammatory cells and their products. Finally, these lesions interfere with the transmission of nerve impulses and lead to neuronal dysfunction (Katsara, & Apostolopoulos., 2018). These inflammatory cells, infiltrating around the nerve, cause demyelination of the myelin sheath and immune attack to myelin basic protein, myelin oligodendrocyte glycoprotein, and proteolipid protein. Macrophages, T-helper type-1 (Th1) cells, Th17 cells, CD8+ T cells, and B-cell secreting autoantibodies are all inflammatory cells that have been found to have a role in MS (Baecher-Allan et al., 2015).

Peripherally activated T cells cross the BBB into the CNS, where they undergo reactivation and release cytokines to exert their effector functions. Th1 cells produce their lineage-specific cytokine, interferon gamma (IFN), in addition to tumor necrosis factor. Th17 cells release their cytokines interleukin (IL)-17, as well as IL-21 and IL-22, and can also express IFN-□, which contributes to their pathogenicity. IL-17 and IFN can also be produced by CD8+ effector T cells. These cytokines lead to the activation of CNS-resident immune cells (such as microglia, astrocytes, and macrophages), as well as to the production of cytokines, increase the antigen-presenting cell function, and increase the production of reactive oxygen species and reactive nitrogen species. Effector T cells can be either regulated in the periphery or in the CNS by FoxP3+ regulatory T cells and by CD8+ Tregs, natural killer cells, and regulatory B cells (Shimizu et al., 2013).

In addition, MS is one of the diseases that show no pathognomonic symptom or sign. It includes a variety of symptoms and signs that shared with other neurological disorders and diagnosis is ultimately based on clinical presentation and exclusion of other possible explanations (Ömerhoca et al., 2018). Clinical history and neurological examination is the cornerstone in MS diagnosis. So, it is very important to identify the clinical attacks that are defined as new neurological insults lasting for 24 h or more, that are not accompanied by fever or infection. It usually recovers completely or partially over 6–8 weeks, either spontaneously, or after treatment with corticosteroids (Jakimovski et al., 2018).

The mainstay of MS diagnosis is MRI. For MS patients, it is regarded as the most significant biomarker for diagnosis and prognosis. For the better and earlier identification of active or chronic lesions, various MRI sequences and methods are available (Thompson et al., 2018). The 2017 McDonald criteria are the most recent iteration of the diagnostic criteria for multiple sclerosis, which have been refined throughout time to include clinical, imaging, and laboratory data (Agah et al., 2018). Identifying a reliable biomarker may accelerate diagnosis of MS and early management of the disease (Cappellano et al., 2021).

A common acidic glycoprotein, osteopontin (OPN) is thought to be an intriguing biomarker due to its involvement in the pathophysiology of a number of illnesses. Recent research has shown that OPN functions through two different methods. The

first is by attracting dangerous inflammatory cells to the lesion site and enhancing their survival. OPN's detrimental effects have been linked to a number of neurologic disorders, including multiple sclerosis, Parkinson's disease, and Alzheimer's disease (Clemente et al., 2016).

MS patients have reported higher levels of OPN and these levels are increased in RRMS than in PP and SPMS, particularly during the relapses. OPN is expressed in reactive astrocytes and microglial cells in patients with RRMS, especially during the relapses (Guzel et al., 2016).

Not only OPN is present abundantly in MS lesions, but also high levels of circulating OPN have been observed in several body fluids such as cerebrospinal fluid, serum, or plasma of MS patients, suggesting that this protein may be targeted as a biomarker to monitor disease activity and disease progression (Cappellano et al., 2021).

Aim

The aim of this study is to evaluate the relationship between osteopontin blood marker and relapse, remission of multiple sclerosis in Saudi Arabia patients in 2024 and correlate it with disease activity Patients and methods

This study is a case–control study, conducted in Immunology Laboratory, Clinical Pathology Department, Prince Muhammad bin Nasser hospital, Gazan, Saudi Arabia. The study was conducted on 60 RRMS patients attending the Outpatient Clinics and Inpatient of Neurology Department of Prince Muhammad bin Nasser hospital, Gazan, Saudi Arabia.

Patients known suffering from other neurological diseases, other chronic inflammatory diseases, tumors, or severe obesity (BMI >40 kg/m^2) were excluded from the study. Patients enrolled in this study were divided into two groups: group I included 30 age-matched and sex-matched individuals as a control group and group II included 60 RRMS patients who were diagnosed according to 2017 McDonald diagnostic criteria [10]. Group II is subdivided into two subgroups: group IIa involved 30 patients in remission and group IIb involved 30 patients in relapse before receiving methyl prednisolone.

All patients were subjected to full history taking, including age, family history, occupation, marital status, disease duration, smoking habit, and drug history in addition to neurological examination using Expanded Disability Status Scale (EDSS) assessment and laboratory investigations after informed written consent approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University, were obtained prior to enrollment. The following laboratory investigations were done for all participants: complete blood count performed on automated cell counter performed on Coulter LH 750 cell counter, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) done on Beckman Coulter AU 480 system (Beckman Coulter Inc., Brea, California, USA), and serum OPN assayed by quantitative sandwich ELISA kit for detection of serum OPN (Bioassay Technology Laboratory, Shanghai, China). It was used according to the manufacturer's instructions with assay range (0.3–90 ng/ml).

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS), version 25.0. Quantitative data were expressed as mean ± SD. Qualitative data were expressed as frequency and percentage. Independent samples t test of significance was used when comparing between two means. χ^2 test was used when comparing between qualitative data. Receiver operating characteristic (ROC) curve was used to detect cutoff value, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. Correlation analysis (using Pearson's method) to assess the strength of association between two quantitative variables, P value less than 0.05, was considered significant.

RESULTS

A total of 90 patients were included in this study. They were classified into two groups: group I, it involved 30 age-matched and sex-matched healthy individuals as a control group, 22 (73.33%) females and eight (26.67%) males. Their ages ranged from 14 to 64 years with the mean ± SD that was 34.13 ± 10.79. Group II involved 60 patients with RRMS who were subdivided into two subgroups: group IIa included 30 patients in remission. They were 26 (86.67%) females and four (13.33%) males. Their ages ranged from 20 to 53 years with the mean ± SD that was 32.2 ± 8.17. And group IIb included 30 patients in relapse before receiving methyl prednisolone. They were 27 (90%) females and three (10%) males. Their ages ranged from 20 to 54 years with the mean ± SD that was 33.4 ± 8.17. All groups were homogeneous in terms of size and

demographic characteristics with no significant differences. But there was a statistically significant higher OPN level in group II than group I ($P < 0.001$) (Table 1).

	Groups			Test of significance		
	Group I Mean±SD	Group IIa Mean±SD	Group IIb Mean±SD	Value	P	Significance
Age	34.13±10.79	32.2±8.17	33.4±8.17	t=0.343	0.711	NS
Sex [n (%)]						
Male	8 (26.67)	4 (13.33)	3 (10)	χ^2=3.36	0.186	NS
Female	22 (73.33)	22 (86.67)	27 (90)			
Marital status [n (%)]						
Single	8 (26.67)	6 (20)	10 (33.33)	χ^2=1.364	0.506	NS
Married	22 (73.33)	24 (80)	20 (66.67)			
Family history for MS						
No	30 (100)	28 (93.33)	27 (90)	Fisher's exact test	0.363	NS
Yes	0	2 (6.67)	3 (10)			
Smoking habit						
No	26 (86.67)	27 (90)	28 (93.33)	Fisher's exact test	0.905	NS
Yes	4 (13.33)	3 (10)	2 (6.67)			
Lymphocyte count ($\times 10^3/mm^3$)	2.5±0.98	1.88±1.07	1.96±1.11	3.019 (F)	0.054	NS
ALT (IU/l)	22.13±13.05	29.53±21.15	25.67±17.59	1.330 (F)	0.270	NS
AST (IU/l)	20.6±8.95	25.73±13.71	23.1±12.09	1.431 (F)	0.245	NS
OPN level (ng/ml)	4.1±1.79	29.48±27.71	23.72±27.6	18.026 (F)	<0.001*	HS

Comparison between group I and group II as regards laboratory data shows a highly significant difference in OPN level ($P < 0.001$) where it shows higher levels in group II. Also, there is a significant difference between both groups in lymphocyte count ($P < 0.05$) as group II demonstrates lower levels of lymphocyte count. But there are no statistically significant differences as regards ALT and AST (Table 2).

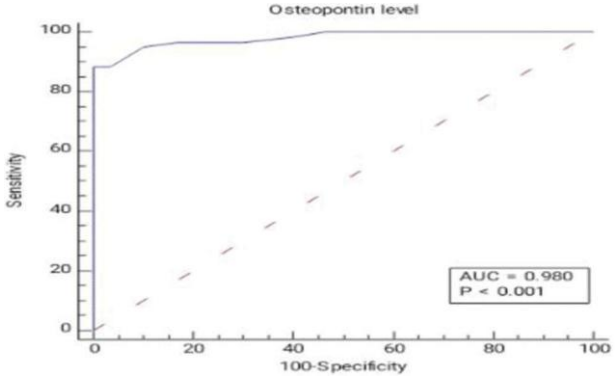
	Groups		Student t test		
	Group I Mean±SD	Group II Mean±SD	t	P	Significance
Lymphocyte ($\times 10^3/mm^3$)	2.5±0.98	1.92±1.08	2.455	0.016	S
ALT (IU/l)	22.13±13.05	27.6±19.38	−1.582	0.118	NS
AST (IU/l)	20.6±8.95	24.42±12.89	−1.636	0.106	NS
OPN level (ng/ml)	4.1±1.79	33.6±27.73	−8.205	<0.001	HS

In group II, there is a positive correlation between OPN and AST ($r = 0.40–0.59$), but there is no correlation with age, EDSS, disease duration, lymphocyte count, or ALT (Table 3).

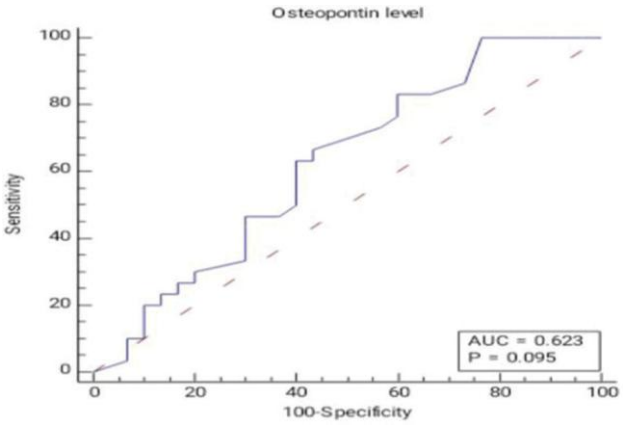
All cases	Age	EDSS	Disease duration	Lymphocyte	ALT	AST
OPN level Pearson correlation	−0.237	0.186	0.174	−0.043	0.073	0.402
Sig. (2-tailed)	0.069	0.156	0.183	0.743	0.578	0.001
Significance	NS	NS	NS	NS	NS	S

Diagnostic performance of osteopontin

Using ROC curve, it was shown that OPN level can be used to discriminate between group I (control) and group II (cases) at a cutoff level of more than 8, with 88.33% sensitivity, 100% specificity, 100% PPV, and 81.1% NPV (Fig. 1).



Using ROC curve, it was shown that OPN level cannot be used to discriminate between group IIa (remission) and group IIb (relapse) (Fig. 2).



DISCUSSION

Myelin sheaths of neurons are destroyed in multiple sclerosis (MS), a complex neurodegenerative disease of the central nervous system. Axonal degeneration, demyelination, astrocyte gliosis, oligodendrocyte loss, and BBB breakdown are the hallmarks of multiple sclerosis (Shimizu et al., 201). MS is characterized by inflammation at every stage, and pro-inflammatory chemokines and cytokines play a key role in the pathogenesis of the disease by destroying the blood-brain barrier, attracting immune cells from the peripheral circulation, and stimulating resident microglia. One of the earliest processes in the formation of MS lesions is believed to be microglia activation. By generating inflammatory cytokines and chemokines, as well as by releasing reactive oxygen species and glutamate, this activation may aid in the advancement of the disease (Agah et al., 2018).

Diagnosis of MS is complex, especially during the early stages in which individuals may present with nonspecific clinical and radiological symptoms. The 2017 McDonald diagnostic criteria state that lesions must meet the spread in time and space requirements in order to distinguish multiple sclerosis from other differential diagnoses. When MS is suspected, MRI and

cerebrospinal fluid investigation for oligoclonal bands can be diagnostic techniques (Zhou et al., 2020).

Numerous bodily tissues and fluids secrete OPN, a highly phosphorylated glycoprotein that plays a part in a number of biological processes, including insulin resistance, wound healing, osteoclast function, and immunological response. Under normal physiological circumstances, OPN is not highly expressed in the central nervous system (CNS); nevertheless, in cases of brain injury or neuroinflammatory diseases, including Alzheimer's disease, Parkinson's disease, traumatic brain injury, stroke, and multiple sclerosis, its expression is elevated (Fischer et al., 2021).

In our study, we found that there was no statistically significant difference among the studied groups as regards age and sex ($P > 0.05$). There was a statistically significant difference between group I and group II as regards lymphocyte count ($P < 0.05$) where group II reported lower levels of lymphocyte count. Various medications used in treatment of MS have an effect on lymphocyte count and may lead to relative and absolute lymphopenia (Fischer et al., 2021). This was approved by Jafarinia M et al., 2020 who described increased neutrophil count and decreased lymphocyte count. However, in our study, there was no correlation between OPN level and lymphocyte count in group II. These results were not in agreement with Carbone et al., 2019 who stated a positive correlation between OPN and lymphocyte count. This may be attributed to underlying disease etiology that was excluded in our study.

In group II, statistical analysis of our study described no significant correlation between OPN level and age, EDSS, or disease duration ($P > 0.05$). These results go in accordance with Jafarinia et al., 2020 who reported no correlation between OPN plasma level and EDSS score, age, and duration of disease. It is contrary to the results reported by Gómez-Santos et al., 2020 who described a positive correlation between serum OPN level and age. This could be referred to accompany another chronic inflammatory disease in Gómez- Santos study.

In our study, we found a significant correlation between OPN level and AST level ($P < 0.05$), but no correlation with ALT. Suri et al., 2021reported significant correlations between the level of OPN and the liver enzymes (AST and ALT). Also, Fouad et al., 2015 revealed significant correlations between OPN and ALT. However, Hodeib et al., 2017 stated no significant correlation. This study had larger sample size and different selection criteria.

OPN levels were significantly higher in patient groups compared with control group ($P < 0.001$). Using ROC curve, it can be used to discriminate between group I and group II at a cutoff level of more than 8 ng/ml, with 88.33% sensitivity, 100% specificity, 100% PPV, and 81.1% NPV. These results are in agreement with Kivisäkk et al., 2014 who reported that OPN levels were higher in relapsing–remitting and SPMS patients compared with healthy controls.

In our study, diagnostic performance of OPN level in discrimination between group IIa and group IIb (remission from relapse) showed that OPN level cannot be used to discriminate between both groups. These results are similar to results reported by Kivisäkk et al., 2014who described no significant association between the OPN levels and disease activity. However, Shimizu et al., 2013 observed significant increase in OPN level during relapse compared with remission. This study involved smaller sample size and included other patterns of MS such as SPMS.

CONCLUSION:

Serum OPN level increased in MS patients. It could be hypothesized that its level increases as a pro-inflammatory biomarker not specific for MS patients. OPN may not be a specific marker for MS. Our data do not support a role for circulating OPN levels as a biomarker for disease activity, but do not rule out a potential role for OPN measurement in the cerebrospinal fluid alone or in association with other biomarkers.

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