Serum osteopontin as a blood biomarker in relapsing–remitting multiple sclerosis Egyptian patients

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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 serum OPN as a blood biomarker in relapsing–remitting multiple-sclerosis (RRMS) Egyptian patients 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.

Keywords:
biomarker, Egypt, multiple sclerosis, osteopontin

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Introduction

Multiple sclerosis (MS) is one of the most important autoimmune-mediated diseases of the central nervous system (CNS). This disease results in inflammation, demyelination, and axonal damage, leading to neurodegenerations [1]. It affects about two to three million people worldwide and is caused by interaction between environmental and genetic factors [2].

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 [3].

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 [4]. 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 [5]. 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-γ), in addition to tumor necrosis factor-α. Th17 cells release their cytokines interleukin (IL)-17, as well as IL-21 and

IL-22, and can also express IFN-γ, which contributes to their pathogenicity. IL-17 and IFN-γ 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 [6].

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 [7]. 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 [8].

MRI is the cornerstone in diagnosis of MS. It is considered the most important diagnostic and prognostic biomarker for MS patients. There are different MRI sequences and techniques for early and better detection of active or chronic lesions [9]. Diagnostic criteria for MS, including clinical, imaging, and laboratory evidences, have been improved over time, with the most recent being the 2017 McDonald criteria [10]. Identifying a reliable biomarker may accelerate diagnosis of MS and early management of the disease [11].

Osteopontin (OPN) is a widely expressed acidic glycoprotein, and is considered as an interesting biomarker because of its role in the pathophysiology of several diseases. Studies in the last years observed that OPN plays its role by two mechanisms: the first mechanism is by recruitment of harmful inflammatory cells to the site of lesion, in addition to increasing their survival. This damaging role of OPN has been documented within the context of various neurologic conditions (i.e., MS, Parkinson's, and Alzheimer's diseases) [12].

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 [13].

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 [11].

Aim

The aim of this study is to evaluate OPN as a blood biomarker in RRMS Egyptian patients and correlate it with disease activity.

Patients and methods

This study is a case–control study, conducted in Immunology Laboratory, Clinical Pathology Department, Ain Shams University Hospitals. The study was conducted on 60 RRMS patients attending the Outpatient Clinics and Inpatient of Neurology Department of Ain Shams University Hospitals. 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).

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).

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).

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).

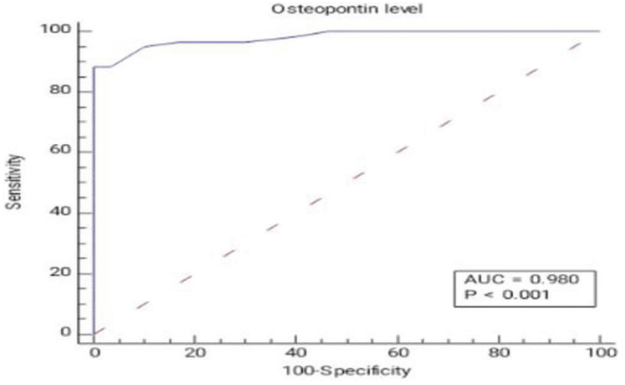
Using ROC curve, it was shown that OPN level cannot be used to discriminate between group IIa (remission) and group IIb (relapse) (Fig. 2).

Discussion

MS is a complicated neurodegenerative disease of the CNS in which the myelin sheaths of the neurons are damaged. MS is characterized by the destruction of BBB followed by oligodendrocyte loss, demyelination, astrocyte gliosis, and axonal degeneration [6]. Inflammation is the characteristic hallmark of MS presenting at all stages, and pro-inflammatory chemokines and cytokines have a crucial role in the pathophysiology of MS by destruction of the BBB, recruiting immune cells from the peripheral circulation and activating resident microglia. It is thought that microglia activation is one of the early events in the development of MS lesions. This activation may contribute to disease progression by producing inflammatory cytokines and chemokines and by releasing reactive oxygen species and glutamate [10].

Diagnosis of MS is challenging, especially during the early stages in which patients may present with nonspecific clinical and radiological signs. According to the 2017 McDonald diagnostic criteria to differentiate MS from other differential diagnoses, lesions need to fulfill the dissemination in time and space criteria. MRI and cerebrospinal fluid analysis for oligoclonal bands can provide diagnostic tools in suspected cases of MS [14].

Figure 1



ROC curve to discriminate between group I and group II as regards OPN. OPN, osteopontin; ROC, receiver operating characteristic.

Table 1 Comparison of sociodemographic and clinical data of all studied groups

	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 (×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

ALT, alanine aminotransferase; AST, aspartate aminotransferase; *F*, Fisher's exact test of significance; OPN, osteopontin; *t*, Student *t* test of significance. *Post-hoc Bonferroni test.

Table 2 Comparison between group I and group II as regards laboratory data

	Groups		Student *t* test		
	Group I Mean±SD	Group II Mean±SD	*t*	*P*	Significance
Lymphocyte (×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

ALT, alanine aminotransferase; AST, aspartate aminotransferase; OPN, osteopontin.

Table 3 Correlation between osteopontin level in group II (remission and relapse) as regards age, Expanded Disability Status Scale, disease duration, lymphocyte count, alanine aminotransferase, and aspartate aminotransferase level

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

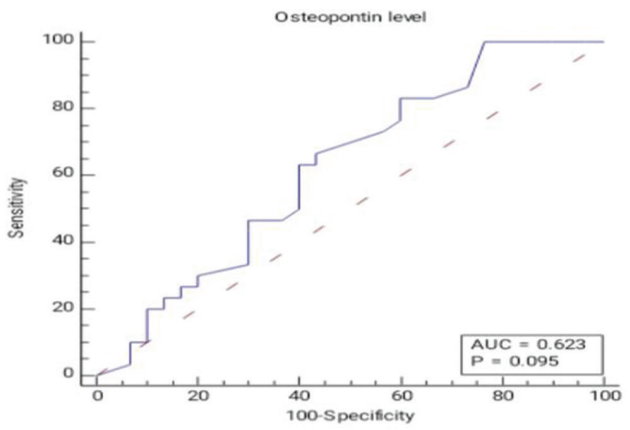
ALT, alanine aminotransferase; AST, aspartate aminotransferase; EDSS, Expanded Disability Status Scale; OPN, osteopontin.

OPN is a highly phosphorylated glycoprotein that is secreted by various body tissues and fluids and has a role in different biological processes such as osteoclast function, wound healing, immune response, and insulin resistance. In the CNS, OPN is weakly expressed under normal physiological conditions, but it shows higher concentration in case of brain injury or neuroinflammatory disease such as Alzheimer's disease, Parkinson's disease, traumatic brain injury, stroke, and MS [15].

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 [16]. This was approved by Jafarinia M *et al.* [17] 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.* [18], 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.* [17] who reported no correlation between OPN plasma level and EDSS

Figure 2



ROC curve to discriminate between group IIa and group IIb as regards OPN. OPN, osteopontin; ROC, receiver operating characteristic.

score, age, and duration of disease. It is contrary to the results reported by Gómez-Santos *et al*. [19], 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*. [20] reported significant correlations between the level of OPN and the liver enzymes (AST and ALT). Also, Fouad *et al*. [21] revealed significant correlations between OPN and ALT. However, Hodeib *et al*. [22] 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*. [23], 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*. [23] who described no significant association between the OPN levels and disease activity. However, Shimizu *et al*. [7] 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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Nil.

Conflicts of interest

There are no conflicts of interest.

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