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Low Serum Myeloperoxidase Levels in Multiple Sclerosis Patients

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Abstract: Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system characterized by inflammation, demyelination, and neurodegeneration. It may lead to physical disability, acute neurological, and cognitive problems. The specific etiology of MS has not been clearly defined to date. One of the key factors that play a role in the pathogenesis of MS is oxidative stress, which increases inflammation and neurodegeneration. Myeloperoxidase (MPO) is one of the enzymes secreted by activated inflammatory cells and is produced by monocytes, macrophages, microglia, and neutrophils. At the same time, myeloperoxidase is one of the components of oxidative stress. MPO has been investigated many times in MS patients, but peripheral blood levels of MPO have been studied very few times. This study investigated serum MPO levels in MS, and the relationship of these levels with patients' age, disease duration, prognosis, annualized relapse rate, expanded disability status scale (EDSS) scores, and disease-modifying drug therapies (DMT) used. The study included 50 MS patients and 50 healthy controls, and their demographic and clinical characteristics were determined. Serum MPO levels were significantly lower in MS patients than in the healthy control group ($p=0.034$). No significant correlation was found between MPO levels and patients' age, EDSS scores, disease duration, DMTs used, and disease progression ($p>0.05$). These results show that low MPO levels in MS patients have an important role in the pathogenesis of MS. There is a need for further studies on this subject. © 2021 NTMS.

Keywords: Multiple Sclerosis; Myeloperoxidase; Oxidative Stress.

1. Introduction

Multiple sclerosis (MS) is an autoimmune, neurodegenerative disease of the central nervous system (CNS) caused by immune dysfunction. It is characterized by demyelination, chronic inflammation, neuronal, and oligodendrocyte loss (1-3). The onset of MS is typically between the ages of 20 and 40 years, it is more common in women and is one of the most important causes of nontraumatic neurological disability in young adults (4). In 1996, the National Multiple Sclerosis Society (NMSS) MS Clinical Research Advisory Committee defined four clinical phenotypes of MS: relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP), and progressive-relapsing (PR) (5). The most common type in more than 80% of MS patients is RRMS, which is characterized by variable neurological symptoms and complete or incomplete recovery during remission. More than half of individuals in the RR stage of MS

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develop the SP form of MS following the accumulation of neurological deficits (6). Although the pathogenesis of MS is still not completely known, it is defined as an inflammatory demyelinating disease, and axonal damage of CNS directly correlates with the intensification of inflammatory processes and oxidative stress (7).

Myeloperoxidase (MPO) is one of the enzymes secreted by activated inflammatory cells such as neutrophils, monocytes, macrophages, and microglia (8, 9). In human neutrophils, it is part of the host defense system against microorganisms. MPO catalyzes the formation of hypochlorous acid (HOCl) and, other cytotoxic oxidants which has powerful activity against a variety of bacteria, viruses, and fungi (9). It produces highly reactive molecules such as hypochlorite, tyrosyl radicals, and aldehydes that can covalently modify lipids, which in turn causes more local tissue damage and increases the inflammatory cascade (10, 11). MPO is found at high levels in active MS plaques in humans (12, 13). However, it has been previously observed that mice devoid of MPO develop experimental autoimmune encephalomyelitis (EAE) and have higher morbidity and mortality compared to their wild-type counterparts (14). Therefore, the role of MPO in inflammatory demyelination remains unclear.

MPO has been studied many times in MS patients, but peripheral blood levels of myeloperoxidase have been studied very few times. This study investigated myeloperoxidase levels in MS patients and healthy control group, and the relationship of these levels with MS patients' age, disease duration, prognosis, annualized relapse rate (ARR), and expanded disability status scale (EDSS) scores.

2. Material and Methods

The study included 50 MS patients aged 18 years and over who were admitted to the Neurology MS outpatient clinic of Ataturk University, Faculty of Medicine between November 6, 2020 and December 21, 2020 and who were diagnosed with MS according to the 2010 Mc Donald diagnostic criteria, and 50 age- and sex-matched healthy controls after obtaining informed consent. Those who received oral or parenteral steroid therapy in the last three months, those with other systemic diseases, MS patients during the attack period, and those who had an attack in the last three months were excluded from the study. The demographic characteristics of the individuals in both groups, MS patients' disease-modifying therapies (DMT), disease duration, the ARR, and disease type were determined. The neurological examinations of MS patients were performed by the same neurologist, and their clinical characteristics and EDSS scores were determined. Venous blood samples were collected from the individuals. Thirty minutes after the collection of blood samples, the samples were centrifuged at 4000 rpm for 10 minutes and serums were taken. The samples were kept at -80 ^{o}C until analysis. Serum MPO levels were measured by the manual method. The test principle of the MPO determination is based on the kinetic measurement of the absorbance at 460 nm wavelength of the yellowish-orange complex formed as a result of oxidation of MPO and o-dianisidine in the presence of H_2O_2. MPO analysis results were calculated as U/mg protein (15). Local ethics committee approval was obtained for our study (09/19/05.11.2020).

2.1. Statistical Analysis

Statistical analysis was carried out using SPSS 22.0 Software package. The normality of data was evaluated by the Kolmogorov-Smirnov test. In comparison of numerical data, the Mann-Whitney U test was used when the number of groups was two for those who did not comply with the normal distribution, and the Kruskal Wallis test was used when the number of groups was more than two. The chi-squared test was used for comparison of categorical variables. Spearman's Rho test was used for correlation analysis of non-normally distributed data. The level of statistical significance was set at $p<0.05$ in all tests.

3. Results

Serum MPO levels were measured in multiple sclerosis patients (n=50) and healthy controls (n=50), and the data were compared. Of MS patients, 70% were female and 30% were male, with a mean age of 36.32 years. Of MS patients, 84% had RRMS and 16% had SPMS. The mean EDSS score of the patients was 2.30±1.34, the mean disease duration was 5.92±4.94 years and the ARR was 0.97±0.49. Of the patients, 18 (36.0%) were using interferon beta-1a, 7 (14.0%) were using interferon beta-1b, 7 (14.0%) were using fingolimod, 6 (12%) were using glatiramer acetate, 5 (10%) were using dimethyl fumarate, 3 (6.0%) were using teriflunomide, and 4 (8.0%) were using natalizumab (Table 1).

The serum MPO levels of MS patients (median=9192.30) were lower than the MPO levels of the healthy control group (median=1076.91) and this decrease was statistically significant (p=0.034) (Table 1). No significant correlation was found between MS patients' serum MPO levels and age, EDDS score, disease duration, ARR (p>0.05) (Table 2). There was no significant difference between the serum MPO levels of MS patients, and the DMT groups used (p=0.558) (Table 2).

When MS patients were classified by disease types, the disease duration and EDSS score of SPMS patients were significantly higher compared to RRMS patients, but there was no significant difference between these two groups in terms of age, ARR and MPO levels (Table 3).

Table 1: Demographic and clinical characteristics and serum myeloperoxidase levels of MS patients and healthy controls.

	MS (n=50)	**Healthy control (n=50)**	**p**
Gender n (%)			
Female	35 (70%)	35 (70%)	1*
Male	15 (30%)	15 (30%)	
Age, years			0.849**
Mean±SD;	36.2±9.4;	36.3±9.8;	
Median(Min-Max)	35 (19-59)	35 (19-57)	
Disease Type n (%)		-	
RRMS	42 (84%)		
SPMS	8 (16%)		
Disease duration, years		-	
Mean±SD;	5.92±4.94;		
Median(Min-Max)	4 (1-22)		
EDSS		-	
Mean±SD;	2.30±1.34;		
Median(Min-Max)	2 (0-6)		
ARR		-	
Mean±SD;	0.97±0.49;		
Median(Min-Max)	0.41 (0.2-1.59)		
DMT n (%)		-	
Interferon beta-1a	18 (36%)		
Interferon beta-1b	7 (14%)		
Glatiramer acetate	6 (12%)		
Fingolimod	7 (14%)		
Dimethyl fumarate	5 (10%)		
Teriflunamide	3 (6%)		
Natalizumab	4 (8%)		
MPO *(U/mg protein)*			
Mean±SD;	8611.22±6688.80;	10303.50±3775.38;	**0.034****
Median(Min-Max)	9192.30 (1038.46-40200)	1076.91 (2076.92-16269.23)	

*****Chi square Test, ******Mann-Whitney U Test, **MS:** Multiple Sclerosis, **RRMS:** Relapsing-remitting MS, **SPMS:** Secondary Progressive MS, **EDSS:** Expanded Disability Status Scale, **ARR:** Annualized relapse rate, **MPO:** Myeloperoxidase, **DMT:** Disease-modifying therapy.

Table 2: Correlation of serum MPO levels with age, EDSS, disease duration, and ARR in MS patients.

		Age	**EDSS**	**Disease duration**	**ARR**	**DMT***
MPO	**r**	-0.046	0.121	0.134	0.208	-
(*U/mg protein)*	**p**	0.651	0.402	0.354	0.147	0.558

Spearman's correlation, * Kruskal Wallis, **MPO:** Myeloperoxidase, **EDSS:** Expanded Disability Status Scale, **ARR:** Annualized relapse rate, **DMT:** Disease-modifying therapy.

Table 3: Comparison of disease type and serum MPO levels, demographic and clinical data in patients with RRMS and SPMS.

	RRMS (n=42)	**SPMS (n=8)**	**p**
Gender n (%)			
Female	31 (%73.8)	4 (%50)	0,178*
Male	11 (%26.2)	4 (%50)	
Age, years			
Mean±SD;	35.69±9.24;	39.62±12.62;	0.450**
Median (Min-Max)	35 (20-57)	39 (19-53)	
Disease duration,years			
Mean±SD;	4.85±4.02;	11.50±5.83;	**0.003****
Median (Min-Max**)**	4 (1-22)	12 (3-20)	
ARR			
Mean±SD;	0.97±0.50;	0,95±0,48;	0.915**
Median (Min-Max)	0.80 (0,42-2,0)	0.90 (0.41-1.60)	
EDSS			
Mean±SD;	1.86±0.89;	4.56±1.01;	**<0.001****
Median (min-max)	2 (0-4)	4.75 (3-6)	
MPO *(ng/ml)*			
Mean±SD;	8589.37±6767.34;	8725.95±6702.51;	0.968**
Median (Min-Max)	9423.07 (1038,46-40200)	7096.14 (1961.53-20192.30)	

*****Chi-squared test, ****** Mann-Whitney U Test, **EDSS:** Expanded Disability Status Scale, **ARR:** Annualized relapse rate, **MPO:** Myeloperoxidase.

4. Discussion

In our study, serum MPO levels were significantly lower in MS patients than in the healthy control group. There was no significant correlation between serum MPO levels and age, disease duration, ARR, EDSS scores in MS patients. Considering disease progression, there was no significant difference between RRMS patients and SPMS patients in terms of MPO levels.

MS is a neuroinflammatory autoimmune disease. In MS, inflammation, demyelination, and axonal damage of both the brain and spinal cord impair physical and cognitive abilities (16**).** In MS, some pathophysiological processes, including chronic inflammation of the CNS, oxidative stress, blood-brain barrier disruption, demyelination, axonal and neuronal damage, and remyelination, are observed (17).

Although the pathogenesis of MS is still not completely known, CNS axonal damage is directly associated with the intensification of inflammatory processes and oxidative stress (7).

Oxidative damage plays a role in cell degeneration in all stages of MS (18-20). Oxidative stress is caused by an imbalance between the production of free radicals and the antioxidant defense system. Increased free radicals, including reactive oxygen species and reactive nitrogen species, cause lipid and protein damage through peroxidation and nitration processes (21). MPO is one of the enzymes secreted by activated inflammatory cells and is produced by monocytes, macrophages, microglia, and neutrophils (8). In human neutrophils, it is part of the host defense system against microorganisms. The main function of MPO lies in the defense of the organism through production of HOCl, a powerful oxidant (9). Among different neurotoxic oxidants in the brain, HOCl is stable, highly reactive, and dominant. This acid plays a role in a number of neurodegenerative diseases, including multiple sclerosis, Parkinson's, and Alzheimer's diseases (22). Myeloperoxidase (MPO) plays a role in MS, with its presence in activated macrophages and an increased risk association of a -463 G/A promoter polymorphism (13). Also, MPO at 17q23.1 is within a region identified in genome scans as a MS susceptibility locus (23,24). In studies conducted considering the potential role of MPO in MS, high levels of MPO have been reported in activated microglia/macrophages at lesion sites in human MS plaques (13, 25). In addition, a significant correlation has been found between high MPO activity, demyelination, neuronal death, and ultimately neurodegeneration (26**).** Similar results have been shown in EAE, an animal model of MS (27-29).

Activated microglia/macrophages secrete MPO which generates cytotoxic HOCl and contributes to the myelin sheath damage surrounding axons (30). Contrary to what was expected in our study, MPO level was low in MS patients. There is evidence in the literature that MPO deficiency may play a role in the pathogenesis of MS. For instance, a study examining the incidence of EAE, an animal model of MS, in MPO knockout mice showed that MPO was detected in activated macrophages in the CNS of wild-type mice, yet unexpectedly, MPO mice had significantly increased incidence of EAE. It was shown that mice devoid of MPO had higher morbidity and mortality compared to their wild-type counterparts, and 90% of MPO

knockout mice developed complete hind limb paralysis 33% of wild-type mice (14). This is the first evidence that MPO plays an important role in EAE, consistent with its putative role in MS. In animals completely devoid of MPO, developmental upregulation of compensatory inflammatory molecules with biological functions different from MPO may play a role, possibly explaining the exacerbation observed in these mice. This concept is supported by the increased proliferation of antigen-specific T cells in mice devoid of MPO with EAE in the same study (14).

In our study, low levels of MPA in the serum of MS patients may play a role in the pathogenesis of MS with a similar mechanism. Similar to these results, considering the potential role of MPO in MS, leukocyte MPO activity has been investigated in patients with different MS subtypes and healthy controls, and low MPO activity has been shown in peripheral blood leukocytes in all MS types compared to healthy controls (31).

Experimental evidence demonstrating the significant degenerative role of MPO in MS disease progression suggests that pharmacological inhibition of extracellular MPO by 4-aminobenzoic acid hydrazide (4-ABAH) attenuates the severity of disease progression in an MS mouse model (26). When the correlation between serum MPO levels and disease progression was analyzed in MS patients in our study, MPO levels were slightly higher in SPMS patients compared to RRMS patients, but this was not statistically significant.

A decrease in myeloperoxidase and oxidative damage biomarker levels has been reported after 14 months of natalizumab treatment in MS patients previously compared to the pre-treatment period (32). In our study, it was investigated whether MPO levels in MS patients differ between DMTs used by patients. However, it is not known whether there is a difference between before and after treatment in our study. This is one of the limitations of our study, together with the small number of patients.

5. Conclusions

In conclusion, low serum MPO levels in MS patients may play a role in the pathogenesis of MS. There is a need for further studies on this subject to eliminate the inconsistency regarding MPO levels in MS.

Conflict of Interests

All authors declared that there is no conflict of interest.

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None

Compliance with Ethical Standards

The study was carried out in accordance with ethical standards in all aspects.

Author Contributions

Bilge N, Yevgi R, Kızıldağ N and Kızıltunç A contributed to the conception and design of the study. Bilge N, Yevgi R, Kızıldağ N and Kızıltunç A contributed to the collection of the data and statistical analysis and evaluation of the results. Bilge N and Yevgi R contributed to the creating and writing of manuscript. Bilge N contributed to revising the work and final approval of the version.

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