

Received: 2015.08.19
Accepted: 2015.11.03
Published: 2016.07.15

Changes in Serum Ceruloplasmin Levels Based on Immunomodulatory Treatments and Melatonin Supplementation in Multiple Sclerosis Patients

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 **Monika Adamczyk-Sowa**
ABCDEF 2 **Paweł Sowa**
BF 1 **Sebastian Mucha**
F 1 **Jacek Zostawa**
B 3 **Bogdan Mazur**
B 1 **Maciej Owczarek**
DG 1 **Krystyna Pierzchała**

1 Department of Neurology in Zabrze, Medical University of Silesia in Katowice, Zabrze, Poland
2 ENT Department, Medical University of Silesia in Katowice, Zabrze, Poland
3 Department of Microbiology and Immunology, Medical University of Silesia in Katowice, Zabrze, Poland

Corresponding Author: Monika Adamczyk-Sowa, e-mail: m.adamczyk.sowa@gmail.com

Source of support: The project was financially supported by the Medical University of Silesia, Poland, grant no. KNW-1-145/K/5/0

Background: The cause of multiple sclerosis (MS) is currently unknown, but it is thought that oxidative damage and iron metabolism mechanisms are involved. The aim of this study was to examine ceruloplasmin concentration in MS patients based on various immunomodifying therapies and to test the effect of antioxidative melatonin on ceruloplasmin levels.





Material/Methods: This prospective study included 102 MS patients and 15 healthy controls. Patients were divided into groups according to different immunomodifying therapies: interferons beta 1a, interferons beta 1b, glatiramer acetate, mitoxantrone, and immunomodifying pre-treatment (A, B, G, Mx, and P groups, respectively), and the relapse R group. MS patients were supplemented with melatonin for 3 months. Serum ceruloplasmin concentrations, EDSS, brain MRI, serum C-reactive protein level, and white blood cell count were examined.

Results: The results indicated significantly increased levels of ceruloplasmin in MS patients. No differences in ceruloplasmin concentrations between the relapse group and controls were observed. In A and G groups, ceruloplasmin levels before and after melatonin were similar to levels in controls. In group B, ceruloplasmin concentration was significantly higher vs. control and relapse groups. After melatonin administration in group B, ceruloplasmin levels decreased. Ceruloplasmin concentrations in the Mx group were significantly higher compared to controls.

Conclusions: We found for the first time that ceruloplasmin concentration in MS patients varies depending on different immunomodulatory treatment and decrease after 3 months of melatonin administration. Ceruloplasmin could be a valuable serum marker for the chronic demyelinating process participating in oxidative stress mechanisms, as well as a neurodegenerative marker, but not a marker of acute-phase MS.

MeSH Keywords: **Ceruloplasmin • Melatonin • Multiple Sclerosis • Multiple Sclerosis, Relapsing-Remitting • Oxidative Stress • Recurrence**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/895702>

 2845  1  4  41



Background

Multiple sclerosis (MS) is an inflammatory-demyelinating and neurodegenerative disease that causes damage to the central nervous system. The incidence is highest in younger women. The main form of MS is relapsing-remitting (RRMS), characterized by occurrence of new symptoms followed by periods of clinical remission. After many years of RRMS, the neurodegenerative process intensifies, as evidenced by lack of relapses and the appearance of the secondary progressive form of MS (SPMS). Neurodegeneration and inflammation are present in all forms of MS, and are morphologically and quantitatively associated with each other [1,2]. Although the cause of MS is currently unknown, recent studies showed that oxidative damage mechanisms are involved [3,4]. Some reports indicate a close relationship between oxidative stress processes and iron metabolism [5,6]. Iron may contribute to the pathogenesis and progression of multiple sclerosis due to its accumulation in the brain, particularly within oligodendrocytes and myelin fibers, and iron contributes to demyelination and neurodegeneration [7]. There are some iron-related proteins involved, such as ferritin, hephaestin, and ceruloplasmin [5].

There are many defense systems against oxidative damage processes. They can be divided into enzymatic and non-enzymatic, as well as endogenous and exogenous. Antioxidants are present in serum, plasma, and erythrocytes. Natural defense mechanisms of tissues against oxidative damage involve an acute-phase reaction in which many serum proteins take part, especially albumin, ceruloplasmin, haptoglobin, and transferrin, which may act as antioxidants. Erythrocytes are a source of catalase (CAT), superoxide dismutases (SODs), glutathione peroxidase (GPx), glutathione S-transferase (GST), and glutathione (GSH) [8].

Ceruloplasmin is one of the main proteins taking part in copper metabolism by distributing it in the human body; it belongs to the group of alpha₂-globulins. One molecule of ceruloplasmin binds 6 atoms of copper. It is responsible for carrying more than 95% of copper in blood serum, with the rest being transported by albumins [9]. Ceruloplasmin, produced mainly in the liver, is also a positive-phase protein, which means its level changes in chronic and acute inflammatory diseases [10,11]. It was reported that ceruloplasmin as an antioxidant can scavenge ROS (reactive oxygen species) [12]. On the cellular level in the central nervous system, ceruloplasmin was detected mainly in human astrocytes, but also in axons and in extracellular space. During the inflammatory process when the blood-brain barrier is damaged, increased ceruloplasmin uptake by astrocytes and axons is observed [5]. There is little research concerning the importance of ceruloplasmin in MS patients and the data are inconsistent. Some authors found that the ceruloplasmin level in MS patients is elevated, particularly during relapse, and decreases after steroid treatment,

but others report no differences in ceruloplasmin concentration in healthy controls and MS patients, both during steroid treatment and without steroids [4,13–16]. Decreased serum ceruloplasmin in MS and other neurological inflammatory-disordered patients was also reported [17].

The antioxidative properties of melatonin have been shown by many authors [18–20] and also by our team [21,22]. However, its potential role in MS treatment remains unclear. Some recent research suggests the role of melatonin in MS course [23]. Therefore, the seasonally-dependent relapse seems to be related to melatonin secretion, depending on the day length [24]. The depression that is very often observed in MS patients may be closely connected to alterations in melatonin and consequent serotonin metabolism [25]. The impact of melatonin on MS treatment was also the subject of interest of our team in recent years, and our results show the beneficial role of melatonin supplementation in this disease [26–30].

Melatonin influences the oxidative status and was found to affect ceruloplasmin levels in experimental protocols of oxidative stress induced in animals, as well as in clinical trials in diabetic patients [31–33].

Our previous reports showed that MS patients have increased oxidative stress markers and that oral supplementation with melatonin acts as an antioxidant [26–30]. Based on this promising information, as well as the conflicting information about the role of ceruloplasmin in MS, we conducted the present research. The aim of our study was to examine the serum concentration of ceruloplasmin acute-phase protein in MS patients based on different immunomodifying therapies. We were also interested in the effect of the potent antioxidant, melatonin, on ceruloplasmin levels. Additionally, we attempted to determine whether there are correlations between serum ceruloplasmin level and C-reactive protein (CRP) concentration or white blood cell (WBC) count.

Material and Methods

Patients

This prospective study was carried out on 117 subjects observed in 2014 in the Department of Neurology in Zabrze, Medical University of Silesia, Poland. We included 102 MS patients diagnosed according to the McDonald criteria (2005) with various forms of disease: RRMS, SPMS, and progressive-relapsing form (PRMS) [34]. The control group consisted of 15 healthy subjects matched for age and sex with the MS patients ($p > 0.05$), but in the Mitoxantrone-treated group age and disease duration were significantly different from others. This exception is a consequence of characteristics of the disease:

SPMS and PRMS form develop from previous RRMS form, so they appear in later life. The male-to-female ratio was similar in all groups and ranged from 1:2 to 1:2.75.

We excluded patients with the following chronic disorders: diabetes; obesity (BMI over 30); hormonal, urinary, or liver abnormalities; infectious or inflammatory diseases; dyslipidemia; and smoking. We also excluded patients taking antioxidative substances, vitamins, or anti-inflammatory medications, as well as those who received hormonal treatment within the last 3 months before the study and those who took sleeping medication in the last 2 weeks before to the study.

Patients were divided into the following groups:

Group C (control group): 15 healthy controls visiting in our Department's ambulatory clinic due to undiagnosed headaches. All control group individuals were considered as healthy and were selected using the exclusion criteria.

Group A (interferons beta 1a-treated RRMS group): 21 patients receiving interferon beta-1a, administered once a week as an intramuscular injection or 3 times a week as a subcutaneous injection.

Group B (interferons beta 1b-treated RRMS group): 23 patients treated with interferon beta-1b, subcutaneously injected every other day.

Group G (glatiramer acetate-treated RRMS group): 11 patients receiving glatiramer acetate daily, subcutaneously injected.

Group Mx (Mitoxantrone-treated MS group): 18 patients with SPMS or PRMS form. All of them received 5 doses of mitoxantrone iv quarterly (12 mg/m²/dose).

Group P (pre-treated RRMS group): 17 patients with *de novo* diagnosed RRMS form, without any MS immunomodifying treatment.

Group R (relapse RRMS group): 12 patients during diagnosed clinical and radiological relapse before steroid therapy.

Demographic characteristics of studied groups are presented in Table 1.

Study protocol

All the procedures were performed after informed consent. Demographic data evaluation, Kurtzke Expanded Disability Status Scale (EDSS), and MRI examinations were performed in all MS patients at the beginning of the study. All MS patients were supplemented with melatonin 5 mg daily for 90 days. Before and after melatonin supplementation, the serum ceruloplasmin concentrations were measured.

Enzymatic assays

Before and after 90 days of melatonin administration, 10 mL samples of venous blood were collected from MS patients

between 6:00 and 7:00 a.m., centrifuged, and frozen until laboratory testing. Blood samples from controls were collected at the beginning of the study. Serum ceruloplasmin concentration was determined according to the method of Richterich [35] based on the fact that ceruloplasmin catalyzes the oxidation of p-phenylenediamine, forming a colored product that can be directly determined by spectrophotometry. The rate of formation of that product is proportional to ceruloplasmin concentration (mg/dl).

C-reactive protein (CRP) and white blood cells (WBC)

Serum concentration of CRP, an acute-phase protein, was measured using the dry chemistry immunological method and a VITROS 250 analyzer (Ortho Clinical Diagnostics, Johnson and Johnson, USA). WBC count was measured in blood using an ADVIA hematology analyzer (model 2120).

Neuroimaging

At the beginning of the study, brain magnetic resonance imaging (MRI) was performed in all MS patients. Imaging was performed with the use of a General Electric HDx 1.5T scanner (USA). Patients were scanned with a standard head protocol (multiple planes, slice thickness 5 mm, contrast media: Gadovist (Gd)) and additional postcontrast 3DT1 sequences (1-mm slice thickness). The scans were evaluated according to the approximate number of supratentorial and infratentorial plaques in T2 images. The number of Gd-enhancing T1 plaques was calculated.

Statistics

The results are expressed as means \pm SD. Normally distributed data were tested with the Kolmogorov-Smirnov test. The Mann-Whitney U test and Wilcoxon test were used for comparisons between groups. Differences between means were considered significant at $p < 0.05$. Correlation analysis was performed using the Pearson test. Results were statistically analyzed using STATISTICA v. 8.0 (StatSoft, Poland).

The study protocol was approved by the Ethics Committee of the Medical University of Silesia (KNW/0022/KB1/130/12).

Results

We found significantly increased levels of serum ceruloplasmin in MS patients without immunomodifying therapy compared to healthy individuals (32.9 ± 8.72 vs. 26.39 ± 5.39 mg/dl; $p = 0.0048$) (Figures 1–4). We found no significant differences in serum ceruloplasmin concentrations between the relapse group (group R) patients and the controls (25.92 ± 6.04 vs. 26.39 ± 5.39 mg/dl; $p > 0.05$).

Table 1. Demographic characteristics of studied subjects.

Group	C	A	B	G	Mx	P	R
Patients number (n) (Total n=117)	15	21	23	11	18	17	12
Age (years)	36.45±8.16	41.22±7.13	39.48±9.42	38.95±10.76	55.74±6.21	37.33±9.23	41.90±7.13
Age significance value (vs. C group)	NA	0.17	0.13	0.56	0.0042	0.84	0.09
Female/Male number (n) (ratio)	11/4 (2.75)	14/7 (2)	16/7 (2.28)	8/3 (2.66)	13/5 (2.6)	12/5 (2.4)	8/4 (2)
EDSS	NA	2.44±0.97	2.47±0.89	3.31±0.77	5.95±1.81	1.37±0.75	3.96±1.98
Disease duration (years)	NA	5.14±3.75	7.13±5.11	4.63±3.09	21.72±14.66	1.45±0.91	6.53±5.13
Treatment duration (months)	NA	21.00±11.95	34.86±14.51	37.55±26.91	19.96±12.43	NA	14.74±11.96
Number of T2 brain MRI lesions	NA	25.88±0.29	26.13±0.91	34.53±0.21	34.69±0.89	23.16±1.71	32.935.11
Number of T1 Gd(+) brain MRI lesions	NA	0.51±0.11	0.64±0.36	0.23±0.25	0	0.38±0.14	1.96±0.83

Presented groups: A (beta-1a interferons treated RRMS group), B (beta-1b interferons treated RRMS group), G (glatiramer acetate treated RRMS group), Mx (mitoxantrone treated SP or PR MS group), P (immunomodifying pre-treated RRMS group), C (control healthy group), R (relapse RRMS group). NA – non applicable. Data was presented as mean ±SD.

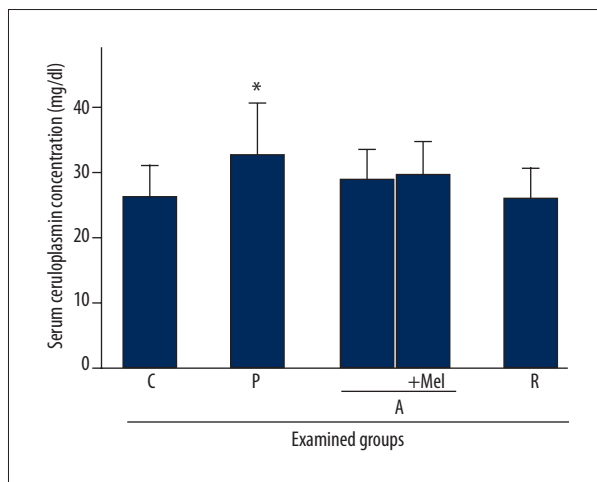


Figure 1. Serum ceruloplasmin concentration in control group (C), immunomodifying MS pre-treated group (P), MS relapse group (R), and in MS INF-beta 1A-treated group (A) before and after 3 months of melatonin treatment (+Mel). Data presented as mean±SD; * p=0.0048 vs. control group.

In group A (interferons beta 1a-treated patients), ceruloplasmin serum levels before and after simultaneous treatment with melatonin were similar (28.58±4.88 vs. 29.59±5.55 mg/dl; p=0.61) and did not significantly differ from the level observed in the control group (p=0.29 and p=0.18, respectively) (Figure 1), and it was similar to that in the pretreated group (p=0.09 and p=0.22, respectively).

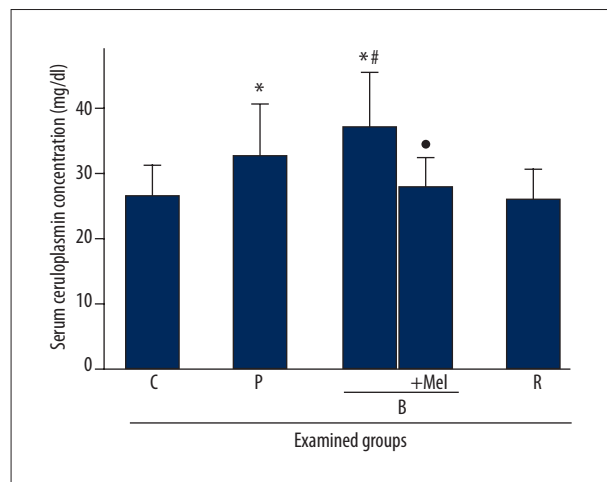


Figure 2. Serum ceruloplasmin concentration in control group (C), immunomodifying MS pre-treated group (P), MS relapse group (R), and in MS INF-beta 1B-treated group (B) before and after 3 months of melatonin treatment (+Mel). Data presented as mean ±SD; * p<0.05 vs. control group; # p<0.05 vs. relapse group; • p<0.05 vs. B group.

In group B (interferons beta 1b-treated patients), serum ceruloplasmin concentration (36.97±8.6 mg/dl) was significantly higher than in the control (p=0.0009) and relapse groups (p=0.001), but it was similar to that in the pretreated group (p=0.067). These levels were also significantly higher compared to group A patients (p=0.001). Interestingly, after 3 months of melatonin administration in group B patients, the levels of ceruloplasmin decreased significantly (36.97±8.6 vs.

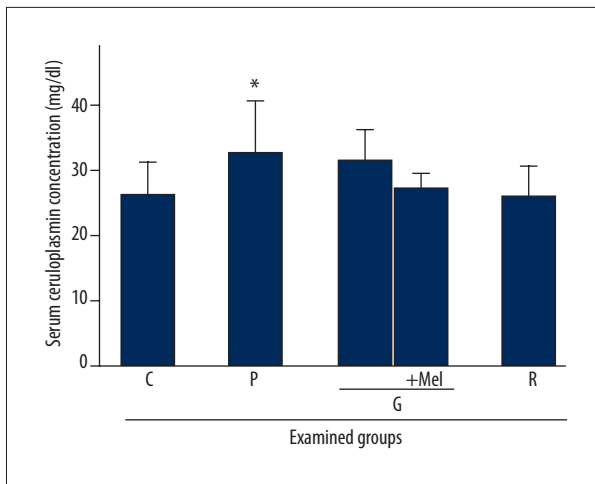


Figure 3. Serum ceruloplasmin concentration in control group (C), immunomodifying MS pre-treated group (P), MS relapse group (R), and in MS glatiramer acetate-treated group (G) before and after 3 months of melatonin treatment (+Mel). Data presented as mean ±SD; * p=0.0048 vs. control group.

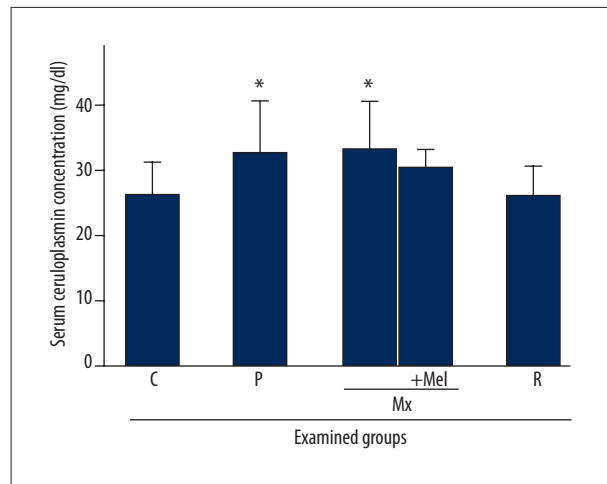


Figure 4. Serum ceruloplasmin concentration in control group (C), immunomodifying MS pre-treated group (P), MS relapse group (R), and in MS mitoxantrone-treated group (Mx) before and after 3 months of melatonin treatment (+Mel). Data presented as mean ±SD; * p<0.05 vs. control group.

Table 2. Serum CRP concentration and WBC count.

Group	C	A	B	G	Mx	P	R
CRP (mg/dL) (reference range 0–5)	0.96± 0.48	2.79± 1.48 ^{a,b,c}	2.89± 1.88 ^{a,b,c}	3.24± 1.67 ^d	5.67± 1.12 ^{d,e,f}	7.52± 1.55 ^{d,e,f}	8.46± 1.32 ^{d,e,f}
WBC (×10 ³ cells/μL) (reference range 5.5–15.5)	6.11± 0.98	7.01± 1.87 ^{a,b}	6.69± 2.51 ^{a,b}	6.81± 1.84 ^{a,b}	9.37± 2.19 ^d	16.01± 3.17 ^{d,e,f,g}	15.98± 4.01 ^{d,e,f,g}

Presented groups: A (beta-1a interferons treated RRMS group), B (beta-1b interferons treated RRMS group), G (glatiramer acetate treated RRMS group), Mx (mitoxantrone treated SP or PR MS group), P (immunomodifying pre-treated RRMS group), C (control healthy group), R (relapse RRMS group). Data was presented as mean±SD. ^a p<0.05 vs. P group; ^b p<0.05 vs. R group; ^c p<0.05 vs. Mx group; ^d p<0.05 vs. C group; ^e p<0.05 vs. A group; ^f p<0.05 vs. B group; ^g p<0.05 vs. G group.

27.63±4.63 mg/dl; p=0.012), achieving levels similar to those in controls (p=0.55) (Figure 2).

Similar to group A, no significant changes were observed between controls, pretreated group, and relapse group, and after melatonin treatment in the group G (glatiramer acetate) patients (26.39±5.39, 32.9±8.72, 25.92±6.04, 27±0.42 vs. 31.26±5.31 mg/dl, respectively; p>0.05) (Figure 3).

Serum ceruloplasmin concentrations in mitoxantrone-treated patients (Mx group) were similar to those in the pretreated group (p=0.89) and were significantly higher compared to controls (32.94±7.35 vs. 26.39±5.39 mg/dl; p=0.03). After melatonin administration in the Mx patient group, the levels of ceruloplasmin decreased slightly, but insignificantly (30.36±1.45 mg/dl) (Figure 4).

The data describing the number of brain MRI changes in the examined groups are presented in Table 1. A strong correlation between the mean number of T2 brain MRI lesions and serum ceruloplasmin concentration was found only in group P (r=0.8, p<0.05).

We also evaluated serum CRP concentration and WBC counts in our patients (Table 2). Significantly higher serum CRP concentrations compared to controls were found in the pretreated MS patients group, relapse group, and Mx-treated group, and these values were elevated above reference levels. Similarly, WBC count in Mx, P, and R groups were higher than in controls, but ranging near the upper limit of reference values. We found no correlation between ceruloplasmin level and CRP concentration or WBC count. No correlations between serum ceruloplasmin concentration and EDSS or disease duration were found.

Discussion

Multiple sclerosis is a demyelinating disease involving inflammatory processes. The cause of the disease remains unknown. Recent studies strongly suggest that an oxidative stress mechanism participates in MS etiopathology [3–6,8,13–16,36]. Our previous reports also supported the presence of increased oxidative stress markers in MS patients [26–30]. Based on this, we are interested in finding specific marker indicating disease progression.

Ceruloplasmin is considered to be a preventive plasma antioxidant participating in iron metabolism and thereby preventing free radical reactions [37]. Additionally, since it was shown in both experimental and clinical studies that MS is strongly connected with iron storage in the brain, ceruloplasmin seems to play an integral role in the pathology of MS [7,38]. Taken together, the findings mentioned above, and the fact that inflammation seems to be the main pathogenetic mechanism in multiple sclerosis, indicate that we should expect ceruloplasmin levels will vary according to disease activity and therapies, but there are few reports in the literature on this topic and the data from different studies are inconsistent. Smith et al. found no differences in ceruloplasmin levels in MS patients with or without steroid therapy compared to controls [16], while others reported a decrease in ceruloplasmin in MS and other inflammatory diseases [17]. Results from our work show that ceruloplasmin serum concentration is elevated in MS patients compared to healthy controls, which can be clearly seen in MS pre-treated individuals. This is in line with our previous studies and expectations, because ceruloplasmin level, as a positive-phase protein, may be increased in chronic and acute inflammatory diseases [11]. Other authors have reported results similar to ours, both in laboratory and clinical studies. It was reported that experimentally-induced oxidative stress is connected with increased serum ceruloplasmin level in rats [31]. In clinical trials, ceruloplasmin concentration in plasma and cerebrospinal fluid was found to be up-regulated in MS patients in comparison to healthy subjects [4,14]. Fiorini et al. emphasized an increase in the ceruloplasmin level during active relapse phase of multiple sclerosis vs. remission [4]. Moreover, steroid treatment was reported to lead to a decrease in ceruloplasmin levels in serum and cerebrospinal fluid [13]. In the present study we did not observe such effects because in our relapse group ceruloplasmin levels were similar to levels in controls.

In the present study, immunomodifying treatment did not induce significant changes in serum ceruloplasmin level compared to the pre-treated group. In the groups treated with mitoxantrone and interferons beta 1b, the levels were significantly higher compared to healthy controls. Interestingly, in patients treated with interferons beta 1b and glatiramer acetate,

ceruloplasmin levels were insignificantly lower compared to the pre-treated MS group, and achieved levels similar to those of the control group. The results of the present study agree with the fact that mitoxantrone generates ROS [39]. Moreover, we previously reported increased oxidative stress parameters in mitoxantrone-treated MS patients [26]. Unfortunately, there is very little data in the literature on the effect of various forms of MS treatment (especially concerning the most recently discovered molecules) on oxidative stress parameters. Therefore, although we previously observed the increased total oxidant status in patients using interferons beta-1b compared to interferons beta-1a- or glatiramer acetate-treated individuals, this matter needs further investigation [29].

The theoretical premises suggest that acute-phase proteins should be useful in monitoring inflammatory diseases; however, the studies prove that in particular diseases not all of them increase to the same extent. In Guillain-Barré syndrome, for example, only haptoglobin, and to a lesser degree, ceruloplasmin, are useful [36]. Ceruloplasmin could be a valuable chronic demyelinating process serum marker connected with oxidative stress mechanisms, as well as a neurodegenerative marker, but not in the acute phase of MS. Interestingly, we noticed that ceruloplasmin levels in immunomodifying pre-treated MS patients were correlated with the number of T2 brain MRI changes. This correlation might be influenced by the iron-related mechanisms described by Hametmer et al. [5], but such a conclusion needs additional confirmation. There have been other attempts to define acute MS markers. It was proposed that cerebrospinal fluid (CSF) chitinase 3-like 1 level may be a characteristic marker of conversion to multiple sclerosis in patients with clinically isolated syndrome [40]; however, considering the routine determination of oligoclonal bands and problems in obtaining CSF samples, use of a serum marker may be preferable.

Although serum ceruloplasmin level was reported to be positively correlated with serum CRP concentration in cardiovascular disease [41], we did not find similar correlations or correlations with WBC count in our MS patients.

The antioxidative properties of melatonin are clearly established, and it is well-accepted that melatonin supplementation during MS treatment benefits disease course and patient quality of life [23,29,30]. In this study we observed antioxidative effects of melatonin after 3-month administration in MS patients, but significant changes in ceruloplasmin level were seen only in the interferons beta-1b group. This seems to be reasonable, because the serum ceruloplasmin levels in the interferons beta-1b group and mitoxantrone group were (similarly to pre-treated patients) significantly higher compared to controls. The similar concentrations of ceruloplasmin in the Mx group, despite melatonin treatment, may be explained by

the slightly higher average age in this group, possibly resulting in higher iron storage in brain tissue.

It appears that melatonin not only defends systems against free radical-induced oxidative stress, but also stimulates erythrocyte antioxidants by increasing antioxidative enzyme activity, as well as affecting the serum antioxidative system by changing ceruloplasmin concentration in MS subjects.

Conclusions

We observed elevated serum ceruloplasmin concentration in *de novo*-diagnosed MS patients. Moreover, the level of serum

ceruloplasmin depends on the kind of immunomodulatory treatment used in MS patients. Higher concentrations of ceruloplasmin were observed in patients treated with interferons beta 1 b and Mx. Interestingly, 3 months of melatonin administration decreased these values to the levels found in healthy controls. We did not observe any serum ceruloplasmin changes during MS relapse. Our results confirm the potential role of ceruloplasmin in MS pathogenesis, but further studies are needed to explore the exact mechanisms involved.

Conflicts of interest

The authors declare they have no conflicts of interest.

References:

1. Frischer JM, Bramow S, Dal-Bianco A et al: The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain*, 2009; 132: 1175–89
2. Mahad DH, Trapp BD, Lassmann H: Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol*, 2015; 14: 183–93
3. Gonet RE: Neurodegeneration in multiple sclerosis: The role of oxidative stress and excitotoxicity. *J Neurol Sci*, 2008; 274: 48–53
4. Fiorini A, Koudriavtseva T, Bucaj E et al: Involvement of oxidative stress in occurrence of relapses in multiple sclerosis: The spectrum of oxidatively modified serum proteins detected by proteomics and redox proteomics analysis. *PLoS One*, 2013; 7: 8: e65184
5. Hametner S, Wimmer I, Haider L et al: Iron and neurodegeneration in the multiple sclerosis brain. *Ann Neurol*, 2013; 74: 848–61
6. Haider L: Inflammation, iron, energy failure, and oxidative stress in the pathogenesis of multiple sclerosis. *Oxid Med Cell Longev*, 2015; 2015: 725370
7. Haider L, Simeonidou C, Steinberger G et al: Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron. *J Neurol Neurosurg Psychiatry*, 2014; 85: 1386–95
8. Gilgun-Sherki Y, Melamed E, Offen D: The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. *J Neurol*, 2004; 251: 261–68
9. Wu X, Liu Z, Guo J et al: Influence of dietary zinc and copper on apparent mineral retention and serum biochemical indicators in young male mink (*Mustela vison*). *Biol Trace Elem Res*, 2015; 165: 59–66
10. Walshe JM: Diagnostic significance of reduced serum caeruloplasmin concentration in neurological disease. *Mov Disord*, 2005; 20: 1658–61
11. Kamanl A, Naziroglu M, Aydılek N, Hacıevliyagil C: Plasma lipid peroxidation and antioxidant levels in patients with RA. *Cell Biochem Funct*, 2004; 22(1): 53–57
12. Lopez-Avila V, Sharpe A, Robinson WH: Determination of ceruloplasmin in human serum by SEC-ICPMS. *Anal Bioanal Chem*, 2006; 386: 180–87
13. Keles MS, Taysi S, Sen N et al: Effect of corticosteroid therapy on serum and CSF malondialdehyde and antioxidant proteins in multiple sclerosis. *Can J Neurol Sci*, 2001; 28: 141–43
14. Hunter MJ, Nleamadim BC, Davidson DL: Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluid from multiple sclerosis patients. *Neurochem Res*, 1985; 10: 1645–52
15. Lamoureux G, Jolicoeur R, Giard N et al: Cerebrospinal fluid proteins in multiple sclerosis. *Neurology*, 1975; 25: 537–46
16. Smith DK, Feldman EB, Feldman DS: Trace element status in multiple sclerosis. *Am J Clin Nutr*, 1989; 50: 136–40
17. Cervellati C, Romani A, Fainardi E et al: Serum ferroxidase activity in patients with multiple sclerosis: A pilot study. *In Vivo*, 2014; 28: 1197–200
18. Beyer CE, Steketee JD, Saphier D: Antioxidant properties of melatonin – an emerging mystery. *Biochem Pharmacol*, 1998; 56: 1265–72
19. Mayo JC, Sainz RM, Antoli I et al: Melatonin regulation of antioxidant gene expression. *Cell Mol Life Sci*, 2002; 59: 1706–13
20. Manchester LC, Coto-Montes A, Boga JA et al: Melatonin: An ancient molecule that makes oxygen metabolically tolerable. *J Pineal Res*, 2015; 59(4): 403–19
21. Zwirska-Korczala K, Adamczyk-Sowa M, Polaniak R et al: Influence of extremely-low-frequency magnetic field on antioxidative melatonin properties in AT478 murine squamous cell carcinoma culture. *Biol Trace Elem Res*, 2004; 102: 227–43
22. Zwirska-Korczala K, Jochem J, Adamczyk-Sowa M et al: Influence of melatonin on cell proliferation, antioxidative enzyme activities and lipid peroxidation in 3T3-L1 preadipocytes – an *in vitro* study. *J Physiol Pharmacol*, 2005; 56: 91–99
23. Anderson G, Rodriguez M: Multiple sclerosis: the role of melatonin and N-acetylserotonin. *Mult Scler Relat Disord*, 2015; 4: 112–23
24. Farez MF, Mascanfroni ID, Méndez-Huergo SP et al: Melatonin contributes to the seasonality of multiple sclerosis relapses. *Cell*, 2015; 162: 1338–52
25. Akpinar Z, Tokgöz S, Gökbel H et al: The association of nocturnal serum melatonin levels with major depression in patients with acute multiple sclerosis. *Psychiatry Res*, 2008; 161: 253–57
26. Adamczyk-Sowa M, Sowa P, Pierzchala K et al: Antioxidative enzymes activity and malondialdehyde concentration during mitoxantrone therapy in multiple sclerosis patients. *J Physiol Pharmacol*, 2012; 63: 683–90
27. Sadowska-Bartosz I, Adamczyk-Sowa M, Galiniak S et al: Oxidative modification of serum proteins in multiple sclerosis. *Neurochem Int*, 2013; 63: 507–16
28. Sadowska-Bartosz I, Adamczyk-Sowa M, Gajewska A, Bartosz G: Oxidative modification of blood serum proteins in multiple sclerosis after interferon or mitoxantrone treatment. *J Neuroimmunol*, 2014; 266: 67–74
29. Adamczyk-Sowa M, Pierzchala K, Sowa P et al: Melatonin acts as antioxidant and improves sleep in MS patients. *Neurochem Res*, 2014; 39: 1585–93
30. Adamczyk-Sowa M, Pierzchala K, Sowa P et al: Influence of melatonin supplementation on serum antioxidative properties and impact of the quality of life in multiple sclerosis patients. *J Physiol Pharmacol*, 2014; 65: 543–50
31. Buyukokuroglu ME, Cemek M, Yurumez Y et al: Antioxidative role of melatonin in organophosphate toxicity in rats. *Cell Biol Toxicol*, 2008; 24: 151–58
32. Cemek M, Büyükokuroglu ME, Hazman O et al: The roles of melatonin and vitamin E plus selenium in prevention of oxidative stress induced by naloxone-precipitated withdrawal in heroin-addicted rats. *Biol Trace Elem Res*, 2011; 142: 55–66
33. Kedziora-Kornatowska K, Szewczyk-Golec K, Kozakiewicz M et al: Melatonin improves oxidative stress parameters measured in the blood of elderly type 2 diabetic patients. *J Pineal Res*, 2009; 46: 333–37
34. Polman CH, Reingold SC, Edan G et al: Diagnostic criteria for multiple sclerosis: 2005 revisions to the “McDonald Criteria”. *Ann Neurol*, 2005; 58: 840–46
35. Richter R: *Chemia kliniczna*. Warszawa: PZWL; 1971 [in Polish]
36. Gutowski NJ, Pinkham JM, Akanmu D et al: Free radicals in inflammatory neurological disease: Increased lipid peroxidation and haptoglobin levels in Guillain Barré syndrome. *Ir J Med Sci*, 1998; 167: 43–46

37. Arosio P, Levi S: Ferritin, iron homeostasis, and oxidative damage. *Free Radic Biol Med*, 2002; 33: 457–63
38. Sands SA, Tsau S, LeVine SM: The habenula and iron metabolism in cerebral mouse models of multiple sclerosis. *Neurosci Lett*, 2015; 606: 204–8
39. Corna G, Santambrogio P, Minotti G, Cairo GJ: Doxorubicin paradoxically protects cardiomyocytes against iron mediated toxicity: Role of reactive oxygen species and ferritin. *Biol Chem*, 2004; 279: 13738–45
40. Comabella M, Fernández M, Martín R et al: Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. *Brain*, 2010; 133: 1082–93
41. Panichi V, Taccola D, Rizza GM et al: Ceruloplasmin and acute phase protein levels are associated with cardiovascular disease in chronic dialysis patients. *J Nephrol*, 2004; 17: 715–20