Acta Med. Okayama, 2024
Vol. 78, No. 3, pp. 259–270


Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

*Original Article*

# The Role of the Lipid Profile and Oxidative Stress in Fatigue, Sleep Disorders and Cognitive Impairment in Patients with Multiple Sclerosis

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**The aim of this study is to investigate the relationship of the lipid profile, dysfunctional high-density lipoprotein, ischaemia-modified albumin and thiol–disulfide homeostasis with cognitive impairment, fatigue and sleep disorders in patients with multiple sclerosis. The cognitive functions of patients were evaluated with the Brief International Cognitive Assessment for Multiple Sclerosis battery. Fatigue was evaluated with the Fatigue Severity Scale and the Fatigue Impact Scale. The Pittsburgh Sleep Quality Index and the Epworth Sleepiness Scale were used to assess patients' sleep disturbance. Peripheral blood samples were collected, and lipid levels and myeloperoxidase and paraoxonase activity were measured. The myeloperoxidase/paraoxonase ratio, which indicates dysfunctional high-density lipoprotein, was calculated. Thiol–disulfide homeostasis and ischaemia-modified albumin were measured.**

**We did not identify any relationship between dysfunctional high-density lipoprotein and the physical disability, cognitive decline, fatigue and sleep problems of multiple sclerosis. Thiol–disulfide homeostasis was associated with cognitive scores. The shift of the balance towards disulfide was accompanied by a decrease in cognitive scores. On the other hand, we did not detect any relationship between fatigue and sleep disorders and thiol–disulfide homeostasis. Our findings revealed a possible correlation between cognitive dysfunction and thiol–disulfide homeostasis in multiple sclerosis patients.**

**Key words:** multiple sclerosis, dysfunctional HDL, thiol–disulfide homeostasis, cognitive decline

Multiple sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS) [1]. It is the most common cause of nontraumatic disability in young adults [2]. Disability in MS is not just physical; it is accompanied by many symptoms that reduce the quality of life of patients, such as cognitive impairment, fatigue, and sleep disorders [3].

Although the neurological findings are attributed to immune-mediated damage to myelin and axons along long tracts, the molecular mechanisms underlying this damage are not fully understood. In recent years, the role of oxidative stress in the development and progression of MS has been the focus of research [4]. On the other hand, a series of changes in lipid metabolism are also believed to play a role in the etiopathogenesis of MS [5]. The latter claim seems possible considering that

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Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

25% of the content of the brain is cholesterol [6], and 70% of this cholesterol is found in myelin structures [7]; in addition, cholesterol plays a role not only in the formation of the myelin sheath but also in intercellular communication [8].

Lipoproteins are essential for the regulation of the inflammatory response and act as mediators of cholesterol transport [9]. Low-density lipoprotein (LDL) particles can be oxidized (ox-LDL) [10]. Ox-LDL induces an inflammatory response by macrophages, dendritic cells and natural killer T cells [11]. High-density lipoprotein (HDL) particles, unlike LDL, have antioxidant and anti-inflammatory properties. HDL reduces the harmful effect of ox-LDL. Some studies have shown that HDL can also be oxidized (ox-HDL) and acquire pro-inflammatory properties. HDL contains paraoxonase 1 (PON), an antioxidant enzyme [12]. Myeloperoxidase (MPO), released from myeloid cells during inflammation, is a prooxidative enzyme. MPO produces hypochlorous acid and peroxynitrite locally, which disrupts the anti-inflammatory capacity of HDL by converting free and bound tyrosine residues into chlorotrosine and 3-nitrotrosine, further inducing a proinflammatory state, and thereby rendering HDL dysfunctional [13]. Therefore, the MPO/PON ratio can indicate the dysfunctional status of HDL.

During inflammation, oxidative stress increases, and HDL, which has antioxidant properties under normal conditions, becomes dysfunctional under oxidative stress. Examining thiol–disulfide homeostasis can indicate the body's oxidative status. Thiols are organic compounds containing a sulfhydryl group. Since they are reductive, they are antioxidant molecules. Reactive oxygen species (ROS) oxidize thiols into disulfide bonds. These disulfide bonds can be transformed back into thiols depending on the oxidant–antioxidant balance of the organism. Thus, there is a dynamic thiol–disulfide balance. A shift of this balance towards disulfide will indicate increased oxidative stress in the body [14].

Ischaemia-modified albumin (IMA) can also provide information regarding the oxidative status. The terminal amino acid of albumin can bind heavy metals. Free radical damage reduces the binding of heavy metals to the N-terminus of albumin. This damaged albumin is called IMA and can be used as an indicator of oxidative stress [15]. Figure 1 summarizes the impact of inflammation and oxidative stress on lipid and albumin levels and the thiol–disulfide balance.

The human brain is highly susceptible to oxidative stress due to the high content of fatty acids that can be

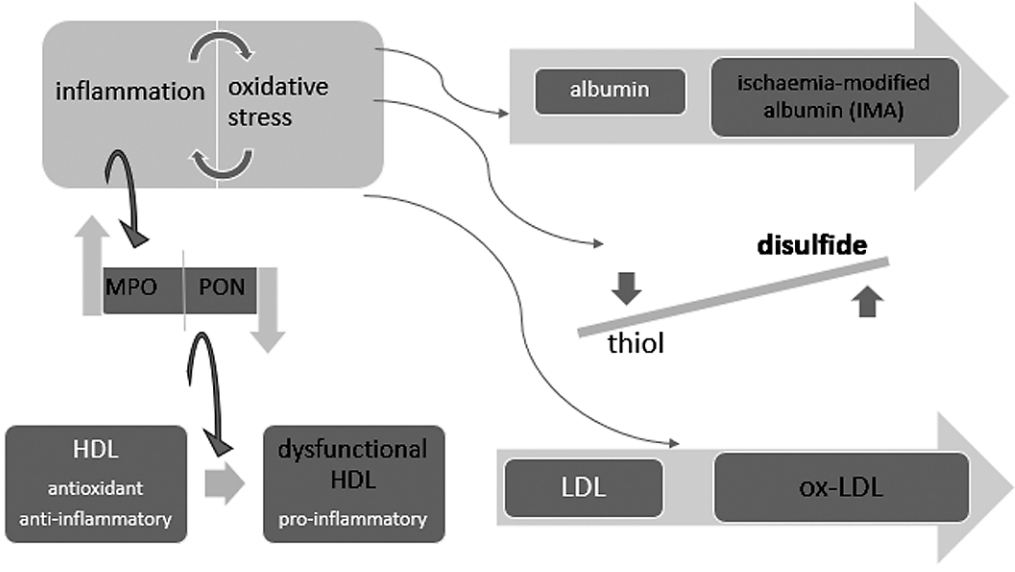


Fig. 1 The impact of inflammation and oxidative stress on lipids, albumin, and thiol–disulfide balance. Inflammation leads to oxidative stress, and oxidative stress leads to inflammation. In the presence of oxidative status, thiol–disulfide homeostasis shifts towards disulfide, IMA increases and LDL becomes oxidized. Inflammation leads to an increase in MPO. Increasing the MPO/PON ratio causes HDL to become dysfunctional. Dysfunctional HDL has lost its antioxidant and anti-inflammatory properties. Such HDL is no longer able to protect against the harmful effects of oxidized LDL.

easily peroxidized. Both cholesterol auto-oxidation products and fatty acid degradation products can trigger neuronal apoptosis in the CNS. Multiple studies have highlighted a strong relationship between increased levels of ROS and lipid peroxidation products in the cerebrospinal fluid and plasma of MS patients and disease progression [16].

The presence of ox-LDL in early and active demyelinating plaques in the postmortem MS brain has been reported [17]. This indicates that LDL oxidation may have an important role in the demyelinating process. HDL, in contrast, has antioxidant properties, and many studies have shown that HDL reduces the harmful effect of ox-LDL, prevents endothelial cell dysfunction and activation, and protects biological membranes [12].

Although there are multiple publications on the relationships between changes in the lipid profile, oxidative stress and clinical parameters of MS, the results are variable, and more studies are needed. In our study, we investigated the relationship of the lipid profile, dysfunctional HDL, IMA and thiol–disulfide homeostasis with cognitive impairment, fatigue and sleep disorders, which impair quality of life at least as much as physical disability in patients with MS but are often outside the scope of standard disability scales.

## Materials and Methods

***Study participants and procedures.*** This study was conducted with 30 healthy volunteers and 65 patients with a diagnosis of MS who were followed in our clinic and met the 2017 Revised McDonald diagnostic criteria. The study was conducted in accordance with the Declaration of Helsinki. Written consent was obtained from all participants and ethics committee approval was obtained from the local ethics committee before the study (E1-21-1847/08.25.2021). Further clinical evaluation of all patients was performed by the researchers. During clinical evaluations, information about the patients' detailed medical history, age, sex, disease duration and treatment information was collected. The patients were examined and their physical disabilities were calculated with Kurtzke's Expanded Disability Status Scale (EDSS). The cognitive functions of the patients were evaluated with the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) battery [18]. This scale includes 3 subtests: the Symbol Digit Modalities Test (SDMT), California Verbal Learning Test (CVLT-II) and Brief Visuospatial Memory Test Revised (BVMT-R). The SDMT tests working memory and information processing speed, the CVLT tests verbal learning, and the BVMT tests visuospatial memory. The presence of depression was assessed using the Beck Depression Inventory (BDI). Fatigue was evaluated with the Fatigue Severity Scale (FSS) and the Fatigue Impact Scale (FIS). The sleep status of the patients was evaluated with the Pittsburgh Sleep Quality Index (PSQI) and the Epworth Sleepiness Scale (ESS).

Following a 12-h fasting period, blood samples were taken and centrifuged at $1,500 \times g$ for 10 min. Serum samples were separated and stored at −80°C until analysis. PON measurement was carried out by spectrophotometric analysis of 4-nitrophenol induced by the enzymatic hydrolysis of paraoxon at a wavelength of 412 nm. For basal PON activity measurement, 50 µL serum, 2 mmol/L paraoxon (O, O-diethyl-Op-nitrophenyl phosphate; Sigma, St. Louis, MO, USA), and 1 mL Tris-hydroxymethyl aminomethane (Tris-HCl) were added to the buffer (100 mmol/L, pH 8). Paraoxon hydrolysis rates were evaluated by measuring 4-nitrophenol formation spectrophotometrically at 25°C and a wavelength of 412 nm. The molar extinction coefficient of 17 100 $M^{-1}$ $cm^{-1}$ was used for PON enzyme activity.

The amount of 1 nm of 4-nitrophenol formed per minute per millilitre of serum was accepted as one unit of PON activity.

Serum MPO activity was measured by a modification of the o-dianisidine method based on kinetic measurement at 460 nm; the rate of yellowish-orange product formation from the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide ($H_2O_2$) was measured. One unit of MPO was defined as the amount degrading 1 µmol of $H_2O_2$ per minute at 25°C. A molar extinction coefficient of $1.13 \times 10^4$ $M^{-1}$ $cm^{-1}$ of oxidized o-dianisidine was used for the calculation. MPO activity was expressed in units per litre of serum.

The MPO/PON ratio was used as an index for dysfunctional HDL measurement, following the method of Haraguchi *et al.* [19].

Serum thiol–disulfide homeostasis was determined by the method developed by Erel *et al.* First, only dynamic and reducible disulfide bonds (-S-S) were reduced to free functional thiol groups (-SH) by sodium borohydride (NaBH4). The remaining unused reductant (NaBH4) was consumed and removed with formaldehyde. Then, all thiol groups, including both reduced

thiols and native thiols, were detected after reaction with DTNB [5,5'-dithiobis-(2-nitrobenzoic) acid]. The native thiol (-SH) and total thiol (-SH + -S-S) concentrations were measured. Half of the difference between the total and native thiols was determined as the dynamic disulfide amount (-S-S). In addition, the -S-S-/-SH, -S-S-/(-SH + -S-S-) and −SH/(-SH + -S-S-) ratios were automatically calculated [14].

The albumin–cobalt binding test was used to detect the presence of IMA. This test was performed by adding 50 mL 0.1% cobalt (II) chloride ($CoCl_2$, $6H_2O$) (Sigma-Aldrich Chemie GmbH Riedstrasse 2, Steinheim, Germany) to the patient serum. After mixing followed by 10 min of incubation to allow for albumin–cobalt binding, 50 mL of 1.5 mg/mL dithiothreitol was added. After mixing followed by 2 min of incubation, 1.0 mL of a 0.9% sodium chloride solution was added to reduce the binding capacity. The blank was prepared similarly with distilled water instead of dithiothreitol. The absorbance of the samples was measured at 470 nm using a spectrophotometer. The results were expressed as absorbance units (ABSU) [20].

Serum HDL, LDL, very-low-density lipoprotein (VLDL), total cholesterol (TC), and triglyceride (TG) levels were measured on the first day of venous blood collection by enzymatic colorimetric testing using an autoanalyzer.

***Statistical analysis.*** The data were evaluated using the statistical package program SPSS Statistics Standard Concurrent User V 26 (IBM Corp., Armonk, NY, USA). Descriptive statistics are expressed as the number of units (n) and percentage (%), mean ± standard deviation, and minimum–maximum values. Normal distribution of the data of numerical variables was evaluated with the Shapiro–Wilk normality test. In comparing two groups, an independent samples *t*-test was used if the data were normally distributed, and the Mann-Whitney *U* test was used if the data were not normally distributed. The relationship between numerical variables was evaluated with the Pearson correlation coefficient if the data were normally distributed or the Spearman correlation coefficient if the data were not normally distributed. A value of $p < 0.05$ was considered statistically significant.

## Results

Sixty-five MS patients, 47 women and 18 men, and 30 healthy volunteers, 21 women and 9 men, participated in the study. The mean age of the patients was 37.1 ± 9.9 years, and the mean age of the healthy controls was 35.9 ± 10.8 years. There was no significant difference between the groups in age or sex distribution ($p = 0.599$ and $p = 0.817$, respectively). The average disease duration was 7.5 ± 5.1 (1-20) years. The mean EDSS score was 2.5 ± 1.5 (0-7.5). The clinical scale scores of the patient and control groups are presented in Table 1. The CVLT and SDMT scores in patients with MS were significantly lower than those in healthy patients, while the BVMT scores were similar. The BDI, FSS, FIS, ESS and PSQI scores were significantly higher in patients with MS.

When the lipid profiles and oxidative status parameters of patients and healthy volunteers were compared, the lipid profiles were similar. MPO activity was increased, PON activity was decreased, and the MPO/PON ratio was significantly higher in individuals with MS compared to healthy individuals ($p = 0.047$, $p = 0.009$, and $p = 0.001$, respectively). While no significant difference in thiol levels was detected between the groups, disulfide levels were found to be high in patients with MS ($p = 0.001$). No difference in IMA levels was detected ($p = 0.809$). The findings are presented in detail in Table 2.

When the relationship among clinical scores, lipid profile and oxidative status was analysed in patients with MS (Table 3), there was a significant positive correlation between disease duration and TG and VLDL levels ($r = 0.295$, $p = 0.017$, and $r = 0.272$, $p = 0.029$, respectively). There was a significant negative correlation between disulfide levels and BVMT score ($r = -0.272$, $p = 0.028$). The SDMT score was negatively correlated with the disulfide/total thiol ratio and positively correlated with the native thiol/total thiol ratio ($r = -0.280$, $p = 0.024$, and $r = 0.299$, $p = 0.016$, respectively).

While the EDSS score was negatively correlated with the CVLT score ($r = -0.260$, $p = 0.036$), it had no correlation with either the fatigue or sleepiness scales. There was a negative correlation between disease duration and CVLT and SDMT scores ($r = -0.473$, $p = 0.001$, and $r = -0.302$, $p = 0.014$, respectively). There was no relationship between sleep or fatigue scores and disease duration. There was a strong positive correlation between EDSS scores and disease duration ($r = 0.396$, $p = 0.001$). Details are shown in Table 4.

**Table 1** Comparison of groups in terms of CVLT, BVMT, SDMT, BDI, FSS, FIS, ESS and PSQI scores

| | Patient (n = 65) M + SD (min-max) | Control group (n = 30) M + SD (min-max) | *P*-value |
|---|---|---|---|
| CVLT | 42.08 ± 12.11 (13–74) | 56.37 ± 9.79 (38–76) | **0.001** ‡ |
| BVMT | 21.37 ± 8.17 (2–36) | 24.27 ± 6.39 (12–34) | 0.090 ‡ |
| SDMT | 34.46 ± 11.34 (5–63) | 43.57 ± 8.57 (28–63) | **0.001** ‡ |
| BDI | 14.46 ± 10.51 (0–43) | 6.6 ± 5.17 (1–21) | **0.001** † |
| FSS | 44.2 ± 13.65 (9–63) | 13.4 ± 5.1 (9–26) | **0.001** † |
| FIS | 86.4 ± 33.19 (7–160) | 17.97 ± 12.95 (3–54) | **0.001** † |
| ESS | 4.89 ± 3.68 (0–17) | 2.47 ± 2.13 (0–7) | **0.001** † |
| PSQI | 7.86 ± 3.87 (0–24) | 2.53 ± 1.78 (0–6) | **0.001** † |

EDSS, Expanded Disability Status Scale; CVLT, California Verbal Learning Test; BVMT, Brief Visuospatial Memory Test; SDMT, Symbol Digit Modalities Test; BDI, Beck Depression Inventory; FSS, Fatigue Severity Scale; FIS, Fatigue Impact Scale; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index. Data are presented as mean (M) ± standard deviation (SD) and minimum-maximum (min-max) values. ‡ *t*-test, † Mann-Whitney *U* test.

**Table 2** Comparison of groups in terms of TC, TG, HDL, LDL, VLDL, MPO, PON, dysfunctional HDL, native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol, and IMA

| | Patient (n = 65) M ± SD | Control group (n = 30) M ± SD | *P*-value |
|---|---|---|---|
| TC (mg/dL) | 182.43 ± 39.62 | 172.87 ± 30.72 | 0.246 ‡ |
| TG (mg/dL) | 115.26 ± 68.71 | 105.97 ± 55.92 | 0.570 † |
| HDL (mg/dL) | 52.03 ± 15.49 | 54.37 ± 14.16 | 0.338 † |
| LDL (mg/dL) | 105.48 ± 32.98 | 97.33 ± 27.09 | 0.241 ‡ |
| VLDL (mg/dL) | 23.43 ± 13.51 | 21.2 ± 11.24 | 0.461 † |
| MPO (ng/mL) | 46.52 ± 25.4 | 35.35 ± 17.95 | **0.047** † |
| PON (U/L) | 245.12 ± 139.29 | 320.97 ± 109.12 | **0.009** † |
| Dysfunctional HDL (MPO/PON) | 0.25 ± 0.16 | 0.12 ± 0.06 | **0.001** † |
| Native Thiol (μmol/L) | 462.28 ± 69.06 | 456.91 ± 22.89 | 0.548 † |
| Total Thiol (μmol/L) | 502.41 ± 71.49 | 491.1 ± 26.63 | 0.404 ‡ |
| Disulfide (μmol/L) | 20.07 ± 2.98 | 16.1 ± 2.66 | **0.001** ‡ |
| Disülfide/Native Thiol | 0.04 ± 0.01 | 0.03 ± 0.01 | **0.001** † |
| Disülfide/Total Thiol | 0.04 ± 0.01 | 0.03 ± 0.01 | **0.001** † |
| Native Thiol/Total Thiol | 0.91 ± 0.01 | 0.93 ± 0.03 | **0.001** † |
| IMA (ABSU) | 0.92 ± 0.07 | 0.93 ± 0.13 | 0.809 ‡ |

HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; TC, total cholesterol; TG, triglyceride; MPO, myeloperoxidase; PON, paraoxonase; IMA, Ischaemia-modified albumin. Results are given as mean (M) ± standard deviation (SD). *P*-value was calculated using ‡ *t*-test or † Mann-Whitney *U* test.

**Table 3** Relationship of the lipid profile and oxidative status markers to the clinical scales

| | | EDSS | Duration of illness | CVLT | BVMT | SDMT | BDI | FSS | FIS | ESS | PSQI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TC (mg/dL) | r | 0.050 | 0.134 | −0.026 | −0.015 | −0.063 | 0.045 | −0.028 | 0.104 | 0.080 | 0.049 |
| | p | 0.694 | 0.288 | 0.837 | 0.905 | 0.615 | 0.724 | 0.823 | 0.408 | 0.527 | 0.698 |
| TG (mg/dL) | r | 0.069 | .295* | −0.074 | −0.075 | −0.175 | 0.018 | −0.006 | −0.124 | 0.097 | 0.050 |
| | p | 0.587 | **0.017** | 0.560 | 0.553 | 0.163 | 0.888 | 0.963 | 0.326 | 0.441 | 0.690 |
| HDL (mg/dL) | r | −0.030 | −0.015 | −0.024 | 0.074 | 0.069 | 0.119 | −0.055 | 0.200 | −0.054 | −0.126 |
| | p | 0.814 | 0.907 | 0.848 | 0.557 | 0.583 | 0.346 | 0.665 | 0.111 | 0.670 | 0.318 |
| LDL (mg/dL) | r | 0.097 | −0.014 | 0.022 | −0.041 | −0.088 | 0.005 | −0.025 | 0.083 | 0.071 | 0.167 |
| | p | 0.443 | 0.913 | 0.863 | 0.746 | 0.486 | 0.971 | 0.846 | 0.512 | 0.575 | 0.184 |
| VLDL (mg/dL) | r | 0.052 | .272* | −0.081 | −0.066 | −0.147 | 0.029 | −0.001 | −0.112 | 0.148 | 0.051 |
| | p | 0.683 | **0.029** | 0.521 | 0.599 | 0.243 | 0.817 | 0.994 | 0.375 | 0.241 | 0.686 |
| MPO (ng/mL) | r | −0.003 | 0.113 | −0.104 | 0.003 | 0.061 | 0.050 | 0.141 | 0.108 | 0.067 | 0.094 |
| | p | 0.982 | 0.370 | 0.411 | 0.980 | 0.631 | 0.690 | 0.261 | 0.392 | 0.594 | 0.457 |
| PON (U/L) | r | 0.089 | 0.044 | 0.132 | 0.159 | 0.121 | 0.062 | −0.030 | 0.003 | 0.089 | 0.178 |
| | p | 0.478 | 0.727 | 0.294 | 0.205 | 0.336 | 0.625 | 0.814 | 0.980 | 0.483 | 0.155 |
| Dysfunctional HDL (MPO/PON) | r | −0.066 | 0.125 | −0.159 | −0.129 | −0.068 | −0.064 | 0.029 | 0.073 | −0.075 | −0.193 |
| | p | 0.600 | 0.320 | 0.206 | 0.307 | 0.591 | 0.614 | 0.819 | 0.562 | 0.550 | 0.124 |
| Native Thiol (μmol/L) | r | −0.058 | −0.118 | 0.032 | −0.133 | 0.075 | 0.035 | −0.114 | −0.061 | −0.048 | −0.162 |
| | p | 0.647 | 0.351 | 0.802 | 0.289 | 0.554 | 0.784 | 0.367 | 0.627 | 0.705 | 0.198 |
| Total Thiol (μmol/L) | r | −0.061 | −0.129 | 0.017 | −0.152 | 0.052 | 0.029 | −0.113 | −0.059 | −0.045 | −0.171 |
| | p | 0.629 | 0.307 | 0.895 | 0.228 | 0.681 | 0.817 | 0.371 | 0.641 | 0.725 | 0.173 |
| Disulfide (μmol/L) | r | −0.061 | −0.182 | −0.167 | −.272* | −0.244 | −0.050 | −0.035 | 0.004 | 0.021 | −0.175 |
| | p | 0.631 | 0.147 | 0.184 | **0.028** | 0.051 | 0.692 | 0.785 | 0.976 | 0.869 | 0.164 |
| Disulfide/ Native Thiol | r | −0.045 | −0.121 | −0.164 | −0.052 | −0.223 | −0.009 | 0.039 | 0.028 | 0.102 | 0.024 |
| | p | 0.720 | 0.336 | 0.191 | 0.678 | 0.074 | 0.942 | 0.758 | 0.826 | 0.417 | 0.848 |
| Disulfide/ Total Thiol | r | 0.050 | 0.013 | −0.217 | −0.106 | −.280* | −0.085 | 0.015 | −0.057 | 0.106 | 0.055 |
| | p | 0.695 | 0.920 | 0.082 | 0.403 | **0.024** | 0.499 | 0.904 | 0.653 | 0.400 | 0.666 |
| Native thiol/ Total Thiol | r | −0.007 | 0.025 | 0.189 | 0.120 | .299* | 0.091 | −0.078 | −0.016 | −0.105 | 0.014 |
| | p | 0.954 | 0.842 | 0.131 | 0.340 | **0.016** | 0.470 | 0.539 | 0.901 | 0.403 | 0.914 |
| IMA (ABSU) | r | 0.149 | 0.172 | 0.005 | 0.238 | 0.096 | 0.102 | −0.069 | 0.028 | 0.088 | −0.039 |
| | p | 0.237 | 0.171 | 0.970 | 0.056 | 0.447 | 0.421 | 0.585 | 0.825 | 0.485 | 0.756 |

HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; TC, total cholesterol; TG, triglyceride; MPO, myeloperoxidase; PON, paraoxonase; IMA, Ischaemia-modified albumin; EDSS, Expanded Disability Status Scale; CVLT, California Verbal Learning Test; BVMT, Brief Visuospatial Memory Test; SDMT, Symbol Digit Modalities Test; BDI, Beck Depression Inventory; FSS, Fatigue Severity Scale; FIS, Fatigue Impact Scale; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index. rho: Spearman correlation coefficient, the bolded digits refer to statistical significance $^{*}p<0.05$ and $^{**}<0.001$.

## Discussion

ROS-mediated tissue damage is critically involved in the inflammatory processes in MS. Activated microglia and macrophages can produce large amounts of oxidizing radicals [21]. A study addressing the relationship between MS lesion formation and progression and oxidative stress has shown that in white matter lesions, MPO, a lysosomal enzyme that produces hypochlorous acid from hydrogen peroxide and chloride anion, is predominantly expressed by macrophages and/or activated microglia [22]. Minahora *et al.* reported that there is a significant increase in MPO levels in patients with MS that is closely related to disability, especially in patients with opticospinal MS [23]. Ramsaransing *et al.* reported that MPO activity is significantly lower in patients with MS. They suggested that high MPO activity may protect against MS by suppressing immunopathological mechanisms [24]. Tasset *et al.* reported that MPO expression was greater in MS patients than in control individuals, but this difference was not statistically significant [25]. In our study, we found that there was a significant increase in MPO levels in MS patients compared to healthy patients, and we did not identify any relationship with disability.

**Table 4** Relationship of the EDSS score and disease duration to cognition, fatigue and sleepiness

| | | EDSS | Duration of illness |
|---|---|---|---|
| CVLT | r | −.260* | −.473** |
| | p | **0.036** | **0.001** |
| BVMT | r | −0.048 | −0.006 |
| | p | 0.704 | 0.964 |
| SDMT | r | −0.218 | −.302* |
| | p | 0.081 | **0.014** |
| BDI | r | −0.066 | 0.038 |
| | p | 0.602 | 0.762 |
| FSS | r | 0.210 | 0.229 |
| | p | 0.094 | 0.067 |
| FIS | r | 0.074 | 0.094 |
| | p | 0.555 | 0.459 |
| ESS | r | −0.063 | 0.102 |
| | p | 0.620 | 0.419 |
| PSQI | r | −0.072 | 0.044 |
| | p | 0.568 | 0.727 |
| EDSS | r | – | 0.396** |
| | p | – | **0.001** |

EDSS, Expanded Disability Status Scale; CVLT, California Verbal Learning Test; BVMT, Brief Visuospatial Memory Test; SDMT, Symbol Digit Modalities Test; BDI, Beck Depression Inventory; FSS, Fatigue Severity Scale; FIS, Fatigue Impact Scale; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index. rho: Spearman correlation coefficient, the bolded digits refer to statistical significance *$p<0.05$ and **$<0.001$.

IMA is also informative about oxidative stress. Cevik *et al.* reported in their study that IMA increased as an indicator of oxidative stress in MS patients [26]. The study by Demirdogen *et al.* showed that IMA levels were similar between patients with MS and healthy individuals [27]. Similarly, in our study, IMA did not differ between patient and healthy groups and was not associated with any clinical parameters.

A number of oxidant molecules are increased and antioxidant molecules are decreased in patients with MS compared to healthy individuals [25]. The complex nature of oxidative and antioxidant mechanisms in MS makes it difficult to interpret changes in these molecules individually.

Thiol–disulfide homeostasis allows for evaluation of the balance between oxidant and antioxidant molecules — that is, the oxidative status — as a whole, rather than measuring individual free oxygen radicals. Therefore thiol–disulfide homeostasis provides more information about the oxidative state. As the oxidative status increases, the balance shifts towards disulfide, and a shift towards thiol indicates good antioxidant defence. Previous studies have shown that thiol–disulfide homeostasis changes in favour of disulfide in MS patients; that is, the balance shifts to the oxidative side [28]. Our study revealed higher disulfide levels in patients with MS. According to our findings, the disulfide/native thiol and disulfide/total thiol ratios were increased, while the native thiol/total thiol ratio was decreased in MS patients, indicating that there was a shift in thiol–disulfide homeostasis towards disulfide. This finding is consistent with the results of previous studies in which separate oxidant and antioxidant molecules were evaluated, indicating the presence of an oxidative state in patients with MS.

In addition to oxidative status, lipid metabolism and clinical effects are being investigated in patients with MS. HDL has anti-inflammatory properties. Increased

HDL appears to have a protective role against blood–brain barrier damage and inflammatory disease activity and has been reported to be associated with positive outcomes in MS [29]. High HDL may reduce cholesterol accumulation resulting from excessive myelin degradation, which has recently been shown to trigger cholesterol crystal formation that promotes inflammation and limits CNS remyelination [30]. However, reports on HDL levels in MS patients are inconsistent. While some studies report an increase in HDL levels [31], others suggest a decrease [32] or show no change [33]. These contradictory findings may be the result of the loss of antioxidant function of HDL in MS patients, suggesting that lipoprotein function may be affected [34]. This anti-inflammatory property of HDL is impaired when the levels of MPO released from leukocytes increase during inflammation in the cellular redox environment, causing HDL to enter a pro-inflammatory state. In other words, in the presence of increased MPO, HDL becomes dysfunctional [13]. It has been shown that leukocyte-derived MPO levels increase in MS patients [22].

In a prospective study with a 5-year follow-up, a positive correlation was observed between the increase in LDL levels and the formation of new T2 lesions, while an increase in HDL showed a negative correlation with the rate of cerebral grey matter atrophy on magnetic resonance imaging (MRI) [35]. Both the studies of Palavra *et al.* and those of Damiza-Detmer *et al.* showed a positive correlation between increased LDL and EDSS [36, 37]. Another study found that the lipid profile had a direct effect on the disability of MS patients [38]. In our study, we did not detect any difference between patients with MS and healthy patients in terms of lipid profiles. We also did not identify any relationship between lipid profiles and disability. On the other hand, MPO activity increased and PON activity decreased in patients with MS. The MPO/PON ratio, a marker of dysfunctional HDL, was increased in patients with MS. This supports the presence of oxidative status in patients with MS. However, we did not detect any relationship between dysfunctional HDL and the physical disability, cognitive decline, fatigue or sleep problems of MS.

MS causes physical and cognitive disability. Cognitive decline is observed in 40-70% of patients [39]. In our study, the CVLT and SDMT scores were low in patients with MS. A low CVLT score was associated with a high EDSS score and long disease duration. The SDMT score did not correlate with the EDSS score, but it did decrease with longer disease duration. The BVMT score was not different between patients and healthy individuals and was unrelated to the EDSS score or disease duration. Although we know from previous reports that cognition may be affected in the very early stages of the disease, it is possible that cognitive disability occurs in parallel with physical disability, which increases with increasing disease duration. Likewise, the underlying processes are the same and lead to cognitive decline as well as physical disability. Previous studies have shown that the SDMT, which measures working memory and information processing speed, is a sensitive test for evaluating cognition in patients with MS [40]. A study of Baetge *et al.* revealed that the combination of the SDMT and BVMT is highly sensitive for detecting cognitive impairment [41]. In a study by Ozturk *et al.*, the SDMT, BVMT and CVLT scores were all improved after MS attack treatment. However, compared with those of the healthy controls, only the BVMT scores were found to be significantly lower, while the SDMT and CVLT scores were similar between the two groups [42]. In contrast, in our study, results for the BVMT, which evaluates visuospatial memory, were similar between MS patients and controls, but the results for the SDMT, which measures information processing speed, and the CVLT, which evaluates verbal learning and memory, were significantly lower in patients. Each of these three tests evaluates a different area and provides unique insight into cognitive functions. That different results were obtained by each study may reflect the nature of the disease, which might affects different areas of the brain to varying degrees.

We found that thiol–disulfide homeostasis was associated with cognitive scores. The shift of the balance towards disulfide was accompanied by a decrease in cognitive scores. There was a negative correlation between the BVMT score and disulfide levels. The SDMT score was negatively correlated with the disulfide/total thiol ratio and positively correlated with the native thiol/total thiol ratio. In other words, the oxidative state, which manifests itself as a shift towards disulfide in thiol–disulfide homeostasis, negatively affects visuospatial memory, working memory and information processing speed; it was not found to be associated with verbal memory. Our findings support the results of Demirdogen *et al.*, who observed a decrease in thiol levels in MS patients with cognitive impairment [27]. However, in a

study investigating thiol–disulfide homeostasis in Alzheimer's disease patients, where oxidative stress is known to play a role in etiopathogenesis, thiol levels were indeed low in patients with dementia, but there was no correlation with cognitive decline [43]. Collectively, then, the results of Demirdogen *et al.* [27] and our own present findings are unique in underscoring the relationship between cognitive impairment and thiol–disulfide homeostasis in patients with MS [27].

While there are multiple studies examining lipid levels in patients with MS, there are fewer studies investigating the relationship between dyslipidaemia and cognition. Noori *et al.* investigated the relationship between cognitive dysfunction and lipid profiles in patients with MS and detected a positive correlation between increased serum LDL and TC levels and cognitive dysfunction. However, they did not detect any difference in cognitive scores between patients with and without dyslipidaemia [44]. We found no relationship between the lipid profile and cognition. Likewise, our study did not detect any correlation between dysfunctional HDL levels and cognitive scores. To our knowledge, this phenomenon has not been studied before. Demirdogen *et al.* reported a negative correlation between IMA levels and cognitive test scores [27], but we did not detect a relationship in our study.

Fatigue affects 75% of MS patients and is associated with a decrease in quality of life [45]. In MRI-based studies of MS patients, the cortico-striato-thalamocortical loop has been associated with fatigue, and it has been suggested that inflammation and neurodegeneration likely underlie the detected radiological abnormalities. The inflammatory mediators TNF-α, IFN-γ, and IL-1β may contribute to the perception of fatigue by leading to inflammation-induced synaptopathy and neurodegeneration. Additionally, abnormal dopaminergic transmission may be associated with MS-related fatigue. Hyperactivation of the hypothalamic–pituitary–adrenal axis, high ACTH levels, and high cortisol levels have also been associated with MS-related fatigue. The contribution of the hypothalamus to the pathophysiology of MS-related fatigue has been supported by MRI studies. Inflammatory cytokines that are effective in treating synaptotoxicity may also have an effect on hypothalamic functions. Other studies suggest that not only central but also peripheral abnormalities, such as those in the peripheral nervous system and skeletal muscles, play a role in the formation or exacerbation of the perception of fatigue in MS patients [46]. The relationships between MS-related fatigue and peripheral inflammatory markers and oxidation have been investigated, but no relationship has been identified [47]. Many medical problems in addition to MS are associated with chronic fatigue. Moreover, it has been suggested that fatigue may be related to each of oxidative stress, oxidative damage to mitochondrial membranes, oxidation of membrane phospholipids and lipid peroxidation. Lipid replacement therapies and antioxidants have been used to increase mitochondrial function in patients with chronic fatigue. Lipid replacement therapies have also been found to be effective at preventing ROS-related changes and reversing loss of function [48].

There is increasing evidence that oxidative stress, more specifically lipid peroxidation, in patients with chronic fatigue syndrome contributes to the disease process and some of the symptoms of the disease [49]. A previous study found that TC, TG, and LDL levels did not differ between patients with chronic fatigue syndrome and controls, but the former had significantly lower HDL levels [50]. However, another study revealed that increased HDL and lower LDL levels, which are associated with improved cardiovascular status, were associated with increased fatigue in healthy population [51].

In a study conducted by Kelly *et al.* in patients with progressive MS, low FSS scores were associated with increased HDL and low TC. Changes in LDL and TG were not associated with decreases in FSS scores [52]. In their study, Browne *et al.* found a relationship between the ratio of TC to HDL and MS fatigue [53]. In our study, we found that the FSS and FIS scores were high in patients with MS, as expected. However, neither lipid profile nor oxidative status was associated with fatigue.

Sleep disorders are common in patients with MS, with a prevalence ranging from 47-62% [54]. Circadian rhythm disorders in MS may result from demyelination of the afferent and/or efferent pathways of the suprachiasmatic nucleus. Fatigue and abnormalities in the circadian sleep–wake rhythm often coexist in MS patients [55]. MRI studies have shown an unexpectedly high incidence of active lesions in the hypothalamus that are likely to affect sleep–wake cycles and fatigue [56]. Hypothalamic MS lesions resulting in low cerebrospinal fluid hypocretin levels have been sug-

gested to cause hypersomnia in affected patients [57]. Dysregulation of melatonin pathways has also been suggested to occur in progressive subtypes of MS [58]. There are case reports of MS patients with REM sleep behaviour disorder, which is suggested to result from a lesion near the pedunculopontine nucleus [59].

Evidence shows that the balance of sleep and wakefulness plays an important role in the production of reactive oxygen and in the regulation of the redox environment of the cell [60]. The quality and quantity of sleep also affect oxidative phosphorylation [61]. Inflammation and oxidative stress play a role in the pathophysiology of inadequate sleep and circadian abnormalities in neuropsychiatric disorders including MS, and sleep disturbance promotes further inflammation, increasing oxidative stress in a vicious cycle [62]. Moreover, sleep is an important resting state with antioxidant properties that are responsible for reducing the oxidative stress produced during wakefulness [63]. Indeed, in MS, inadequate sleep can increase oxidative stress and worsen disease severity [64]. There have been only a limited number of publications on the relationship between sleep and lipid metabolism. In one, delayed sleep timing and longer nighttime sleep duration were associated with a greater risk of dyslipidaemia in a rural cohort study with 1,427 participants [65]. In another, continuous positive airway pressure treatment was reported to improve the lipid profile in patients with obstructive sleep apnea syndrome [66]. A third study reported that both short and long sleep durations were associated with the risk of dyslipidaemia [67]. In our present study, we found that the BDI, ESS and PSQI scores were high in patients with MS, indicating that they were depressed and their sleep quality was poor. However, neither the lipid profile nor oxidative status was associated with sleep disorders, and physical disability and disease duration were not associated with either sleep disorders or fatigue.

Although lipid metabolism changes and oxidative stress seem to play a role in the etiopathogenesis of MS, their one-to-one correlations with clinical symptoms and signs of MS, such as physical and cognitive disability, fatigue, and sleep disorders, have not been demonstrated in a consistent and reproducible manner. Physical and cognitive disability, fatigue and sleep disorders are multifactorial signs and symptoms that mainly involve the loss of myelin and axons in relevant regions and associated networks of the CNS. It is thus difficult to explain the many possible changes using only instantaneous lipid measurements and individual measurements of oxidative stress markers.

The results of our study emphasize that the presence of dyslipidaemia is not an invariable finding in patients with MS. Our analysis of peripheral blood lipid levels did not reveal the relationship between lipid metabolism and the immune system suggested by multiple previous studies and hypothesized herein. Lipid levels were also not found to be associated with cognition, fatigue or sleep disorders. Although signs of dysfunction of the anti-inflammatory and antioxidant HDL under oxidative stress have been reported, this dysfunction has no effect on disability, cognition, fatigue, or sleep disturbance.

The existence of an oxidative state was revealed in our study, as in previous studies. Thiol–disulfide homeostasis seems to provide substantial information about the oxidative state. In contrast, IMA does not appear to be an oxidative marker in patients with MS. Thus, thiol–disulfide homeostasis, which can be measured instead of a series of separate oxidative marker measurements, provides more consistent information about overall oxidative status. Additionally, we found that a shift in thiol–disulfide homeostasis to the oxidant side was associated with cognitive impairment according to the SDMT and BVMT but not the CVLT. Each of these tests measures the functions of anatomically different brain regions. Since MRI findings were not included in our study, it is not possible for us to draw conclusions on the effect of oxidative stress. Therefore, these findings need to be confirmed by other studies that include neuroimaging. Nonetheless, our study is one of the few analyses of the relationship between cognition and thiol–disulfide homeostasis. It is also the first investigation into the relationship between thiol–disulfide homeostasis and fatigue and sleep disorders in patients with MS, although no relationship was revealed.

Both lipid metabolism and oxidative status are dynamic processes. Along with instant measurements, changes in lipid levels and oxidative status, which improve with medical interventions, should also be evaluated to determine whether these changes affect clinical scores during long-term follow-up.

The nature of cognition, fatigue, and sleep disturbance in MS patients is complex and multifactorial. Although oxidative stress and lipid metabolism abnormalities are believed to be responsible for the etiopatho-

genesis of MS, it is difficult to explain the emergence of symptoms solely through these factors. MS is an inflammatory demyelinating and subsequently neurodegenerative disease, and the abnormalities in both oxidative stress and lipid metabolism reported in previous reports are not trivial; however, consistent reproducible results need to be obtained before assessing its impact on clinical parameters. Given the heterogeneity of MS, further research is needed, including analysis of the impact of the number and distribution of MS lesions on cognition, fatigue, and sleep disturbance.

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