



OPEN The impact of oxidative stress on symptoms associated with multiple sclerosis

Raquel Piñar-Morales^{1,2}✉, Raquel Durán^{2,3,4}, Alicia Bautista-García^{3,4}, Maria Jose García-Mansilla⁴, Pablo Aliaga-Gaspar⁵, Francisco Vives-Montero^{2,3,4} & Francisco J. Barrero-Hernández^{1,2}

Multiple sclerosis (MS) is a chronic, inflammatory and degenerative disease affecting the central nervous system, the pathophysiology of which is unknown. Oxidative stress appears to play a significant role in the initiation and the perpetuation of the inflammatory and neurodegenerative processes associated with MS. We have analysed parameters relating to oxidative stress in people with multiple sclerosis (pwMS) and in healthy individuals. Superoxide dismutase (SOD) activity was higher in pwMS than the control group. Meanwhile, total antioxidant capacity (TAC), 4-hydroxynonenal (4-HNE) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) were lower in pwMS. The TAC levels correlated with age and disease duration. Regarding the relationship with disease activity, SOD levels were related to T2-FLAIR lesion burden in MRI and 8-OHdG to annualised relapse rate. PwMS with slower processing speeds exhibited lower levels of TAC and higher 4-HNE levels. No relationship was found between oxidative stress and the presence of depression, fatigue or quality of life. These results link oxidative stress to the pathogenesis of MS. Our findings suggest that these parameters might vary in function of disease activity or be related to the presence of MS-associated symptoms such as CI.

Keywords Cognitive impairment, Multiple sclerosis, Oxidative stress, Superoxide dismutase, Total antioxidant capacity, 4-hydroxynonenal

Multiple sclerosis (MS) is a chronic inflammatory, autoimmune and neurodegenerative disease affecting the central nervous system (CNS). Inflammation, demyelination and axonal loss develop and are expressed from the early stages of the disease¹. The most common type is relapsing-remitting MS (RRMS), which is present in 85% of patients, and is characterized by relapses involving vision loss, sensory disturbance or ataxia, among other symptoms, with variable degree of recovery. The progressive types are less prevalent and involve the progressive worsening of disability². In both types, symptoms such as fatigue, sphincter dysfunction, depression and cognitive impairment (CI) are common and greatly affect patients' quality of life.

The mechanisms of axonal damage, tissue injury and neurodegeneration in MS are not well understood, but evidence regarding the involvement of oxidative stress is growing. Various cell types³ and central and peripheral mechanisms come into play, generating, among other, reactive oxygen species (ROS) and reactive nitrogen species (RNS)⁴. These components are generated physiologically and can also be caused by the presence of pathogens, certain cytokines or radiation. Tobacco consumption appears to play a significant role in oxidative stress, particularly among people with multiple sclerosis (pwMS). Not only does its consumption increase the risk of developing MS, it could also influence the progression of the disease. This influence may be at least partly related to oxidative stress, since smoking habit decreases factors related to antioxidant capacity, such as uric acid⁵. In moderate amounts, oxidative stress have a regulatory effect at various levels, but they are highly reactive and have the capacity to degrade proteins, lipids and DNA. At the cellular level, there are mechanisms in place to maintain homeostasis and avoid the harmful effects of excess cellular oxygen; these include cofactors such as copper or zinc, antioxidants like vitamin C, the Nrf2-Keap1-ARE pathway and enzymes such as superoxide

¹Neurology Department, Hospital Universitario Clínico San Cecilio, Granada, Spain. ²University of Granada, Instituto de Investigación Biosanitaria IBS, Granada, Spain. ³Department of Physiology, Faculty of Medicine, University of Granada, Granada, Spain. ⁴Institute of Neurosciences "Federico Olóriz", Centre of Biomedical Research Centre, University of Granada, Granada, Spain. ⁵Neuroimmunology and Neuroinflammation Group, Instituto de Investigación Biomédica de Málaga (IBIMA), Facultad de Medicina, Unidad de Gestión Clínica (UGC), Neurociencias, Hospital Regional Universitario, Universidad de Málaga, Málaga, Spain. ✉email: rpinararmorales@gmail.com

dismutase (SOD), catalase or glutathione peroxidase⁶. When oxidation exceeds the antioxidant mechanisms, oxidative stress occurs resulting in damaged proteins, DNA and lipids, and which can even cause cell death⁴.

Inflammation is the initial phenomenon that takes place in MS, triggering, among others, neurodegenerative phenomena independent of demyelination⁷. It is not just inflammation that is involved in the development of neurodegeneration, but also several factors that interact amongst themselves and that include mitochondrial damage, which is closely related to oxidative stress^{8,9}. The importance of oxidative stress in MS is not limited only to the genesis of brain injury, it is also related to blood-brain barrier permeability¹⁰ and the immune response, given that the various subtypes of T lymphocytes exhibit a susceptibility to and resistance against ROS¹¹. In both in MS and other neurodegenerative processes, the release of ROS by the innate immune cells appears to play a prominent role in the development and perpetuation of the neurodegenerative process¹².

Various studies have successfully demonstrated the presence of lipids, proteins and oxidised DNA in the brain tissue of people with pwMS, in both the relapse-remitting and progressive types^{4,13}. The presence of these components is greater in the classic acute active lesions associated with the relapsing-remitting type in comparison to the slowly expanding lesions (SELs)⁴ and lesser in inactive lesions and normal-appearing white matter¹⁴. Various studies have shown promising results when measuring serum, plasma or cerebrospinal fluid (CSF) levels of enzymes and molecules involved in the antioxidant mechanisms or products derived from oxidative stress^{15,16}. However, it has not yet been possible to establish their role as biomarkers due to the heterogeneity of the methodology used and the widely varying results¹⁷. Moreover, the studies linking oxidative stress to MS symptoms such as fatigue, depression or cognitive impairment (CI), are scarce. In our paper, we aim to determine serum levels of markers for oxidative stress in pwMS by examining parameters of antioxidant capacity measured in different ways and through various pathways, as well as products derived from oxidative stress in lipids and DNA. We also aim to assess the differences with healthy controls. Furthermore, we aim to correlate these results with other demographic, clinical, neuroimaging and quality of life variables, as well as the presence of associated symptoms, such as fatigue, depression and cognitive impairment.

Materials and methods

Study population

A cross-sectional observational case-control study was conducted on pwMS with a diagnosis of MS according to the 2017 McDonald criteria in the period between April 2020 and April 2021. These individuals were being monitored by the demyelinating diseases clinic at San Cecilio Clinical University Hospital. The pwMS included were over the age of 18, exhibited relapsing types of MS and did not exhibit any inflammatory systemic or neurological, infectious or degenerative diseases that the researchers believed could influence the results. PwMS with primary progressive types of the disease were excluded. The control group comprised healthy individuals with no known neurological or systemic diseases.

Demographic and clinical variables

Demographic variables, such as age, gender, level of education and the existence of a smoking status, were collected. Clinical variables obtained were age at disease onset, location of the first relapse, annual relapse rate (ARR) in the year before inclusion in the study, corticosteroids treatment in the six months prior, MS evolution time and expanded disability status scale score (EDSS). In addition, processing speed was evaluated using the symbol digit modality test (SDMT), walking speed using the 25-Foot Walking Test (25WFT)¹⁸ and fine manual dexterity using nine hole peg test (9HPT)¹⁹. Scales were applied to assess fatigue, depression and quality of life. The Modified Fatigue Impact Scale (MFIS) was used to evaluate the presence of fatigue²⁰. The Beck's Depression Inventory (BDI-II) was also administered to determine the presence and degree of depression²¹ and quality of life was assessed using the visual analogue scale of the EuroQol-5D, which ranges from 0 (worst state of health imaginable) to 100 (highest state of health)²².

To measure the lesion load, the people who had undergone a brain MRI scan in the year prior to inclusion in the study were analysed. The number of T2 lesions and the gadolinium-enhancing lesions were recorded.

Regarding disease modifying treatment (DMT), the current DMT and the exposure time were recorded, as well as the number of DMTs previously used.

Biochemical and molecular variables

For each pwMS, the levels of vitamin D, ferritin and uric acid were analysed. SOD activity, total antioxidant capacity (TAC) and glutathione reductase (GR) activity were established as markers of the antioxidant capacity. Serum levels of 4-Hydroxynonenal (4-HNE) and 8-hydroxy 2 deoxyguanosine (8-OHdG) were measured to evaluate oxidative stress. In the control group, SOD, TAC, GR, 4-HNE and 8-OHdG were analysed.

Blood samples were drawn from the cubital vein into EDTA tubes. Plasma was isolated by centrifugation for 15 min at 3,000 rpm and stored at -80°C until analysis. Total protein amount was measured using a Bicinchoninic acid (BCA) protein assay kit (Pierce BCA Protein Assay Kit, ref. 23227, Thermo Scientific, Madrid, Spain) according to the manufacturer's instructions.

4-HNE and 8-OHdG levels, as well as SOD and GR, were measured as indicators of oxidative stress by commercial assay kits [4-HNE ELISA Kit- E-EL-0128; 8-hydroxy 2 deoxyguanosine ELISA Kit-ab201734; Superoxide Dismutase Colorimetric Activity Kit-EIASODC; OxiSelect™ Glutathione Reductase Assay Kit-STA-812, respectively]. TAC was also analysed as a direct measurement of the total antioxidant capacity of biomolecules from plasma to reduce copper II to copper I, according to the manufacturer's protocol (OxiSelect™ Total Antioxidant Capacity-STA-360). All samples were analysed in duplicate. Results were expressed as nanograms/mL (ng/mL) of 4-HNE, ng/mL of 8-OHdG, units of fluorescence/mL (U/mL) of SOD, microunits/mL (mU/mL) of GR and millimolar (mM) of antioxidant concentration.

Statistical analysis

Exploratory data analysis was conducted using density plots and Q-plots. Furthermore, the normality of the variables of interest was assessed using the Shapiro-Wilk test. As the data exhibited a non-normal distribution, non-parametric tests were used. Specifically, the Mann-Whitney U test was employed. Boxplots were used to present this analysis.

The correlation between quantitative variables was evaluated using the Spearman correlation coefficient, and the results were visually depicted using scatter plots. Additionally, the biserial or polychoric correlation coefficients were employed to explore relationships between quantitative and categorical variables. To study the joint linear relationship of different variables with oxidative stress variables, complementing the correlation analysis, a multiple linear regression model was performed for each oxidative stress variable in R.

All analyses were performed using version 4.0.5 of the R software.

Ethics declarations

In order to participate in this project, all participants were required to fill out an informed consent form in writing. Participation therein was voluntary. All the records were codified, thus guaranteeing compliance with the Organic Law on the Protection of Personal Data (Law 15/1999). Data were collected by the unit supervisors during the scheduled check-ups according to the usual clinical practice. The project was approved by the hospital's Biomedical Research Ethics Committee CEIM/CEI Provincial of Granada with the code 1112-N-20. All research was performed in accordance with relevant guidelines/regulations and has been performed in accordance with the Declaration of Helsinki.

Results

Demographic and clinical variables

The study included 77 pwMS, with an average age of 44.4 ± 10.8 years. Of these, 67.5% (52) were women. The control group ($n = 42$) had an average age of 45.9 ± 14.89 years, and 69.1% were women. No significant differences were observed between the two groups.

Demographic and clinical characteristics of the pwMS are shown in Tables 1 and 2. In the year prior to the study, 60 patients (79%) had exhibited no relapse, 15 (19.48%) had had one relapse and 2 (2.69%) had two relapses, and five patients (6.5%) underwent treatment with corticosteroids in the six months prior to inclusion.

The distribution of DMT among the pwMS was as follows: dimethyl fumarate (DMF) in 24 patients (32.43%); interferon beta (IFN) in 16 patients (21.62%); fingolimod (FTY) in 9 (12.16%); glatiramer acetate (GA) in 8 (10.81%); (NTZ) in 5 (6.75%); alemtuzumab (AZM) in 4 (5.40%); ocrelizumab (OCR) in 3 (4.05%); teriflunomide (TERI) in 3 patients (4.05%) and cladribine (CLA) in 2 (2.70%).

According to the MFIS, fatigue was exhibited by 40 patients (55.5%). Slowing in the speed of processing, as measured by the SDMT, was shown by 37 patients (48.64%) and some degree of depression was indicated by 26 patients (34.66%) in the BDI-II.

Variables of oxidative stress

The values of oxidative stress in the pwMS and controls, pwMS smokers vs. non-smokers and pwMS active and non-active are shown in Fig. 1. SOD activity was higher in pwMS than in the control group, while TAC, 4-HNE and 8-OHdG were lower in pwMS. There were no differences in the GR activity.

With respect to the characteristics of the disease, the TAC levels were higher in pwMS with more time of evolution ($r = 0.232$, $p = 0.047$). 8-OHdG levels were slightly higher in pwMS who had experienced at least one relapse in the previous year ($p = 0.023$). Regarding other analytical parameters studied, 8-OHdG levels correlated with ferritin ($r = 0.301$, $p = 0.017$) and uric acid ($r = 0.377$, $p = 0.003$). No differences were observed based on other clinical and epidemiological variables, or those related to current or past DMT. The study revealed no differences among patients in terms of fatigue, quality of life, or depression levels. The pwMS sample was divided into those treated with high-efficacy DMT (AZM, CLA, FTY, OCR, NTZ) and those treated with moderate-efficacy DMT (GA, DMF, IFN, TERI). No differences were found between the two groups (Table 3). Multiple linear regression analysis revealed a positive linear correlation was observed between MRI lesions and SOD levels, as well as between EDSS and GR (Table 4).

Variables relating to cognitive impairment

We performed an additional analysis of the epidemiological, clinical and oxidative stress data in relation to the SMDT score and the presence of CI, setting a cut-off point of 38 as a cut-off point (≤ 38 with CI, > 38 without CI)²³. We found that CI was related to age ($p < 0.001$), level of education ($p = 0.006$), time of evolution ($p = 0.001$), EDSS ($p < 0.001$), score in 9HPT ($p < 0.001$) and in 25WFT ($p < 0.001$). Regarding other disease-related factors, the presence of CI was associated with a higher MFIS score ($p = 0.006$) and a poorer quality of life ($p < 0.001$) (Table 5). The CI did not correlate with other epidemiological, clinical or treatment-related variables (data not shown).

The 4-HNE levels were correlated with slowing of processing speed ($p = 0.042$), with signs of statistical significance in the case of the TAC levels (0.072). The SDMT score correlated positively with the levels of 4-HNE ($r = 0.312$, $p = 0.022$) and negatively with the levels of TAC ($r = -0.285$, $p = 0.015$) (Fig. 2).

Discussion

Oxidative stress plays a prominent role in the pathophysiology of MS. However, different studies of oxidative stress in pwMS have shown equivocal results so far. In this paper, we have analysed the values of antioxidant agents (SOD and GR activity, and TAC levels) and of products of lipid and DNA degradation (4-HNE and

	pwMS (n = 77)
Age (years) ^a	
Educational level	44.24±10.81
Universitary n (%)	28 (36.4%)
Primary n (%)	26 (33.8%)
Secondary n (%)	23 (29.9%)
Smoking status	
Non-smokers n (%)	50 (64.7%)
Smokers n (%)	37 (35.1%)
First relapse localization	
Spinal cord n (%)	20 (26%)
Sensitive n (%)	16 (20.8%)
Optic neuritis n (%)	18 (23.4%)
Brainstem n (%)	15 (19.5%)
Motor n (%)	2 (2.6%)
Multifocal n (%)	2 (2.6%)
Others n (%)	4 (5.2%)
Age first symptom (years) ^a	29.96±10.4
Disease duration (years) ^a	14.25±10.5
EDSS (median) ^a	2
Categoric EDSS	
<2 n (%)	38 (49.4%)
2–5 n (%)	34 (44.2%)
>5 n (%)	5 (6.5%)
ARR previous year ^a	0.24±0.49
9HPT D (seconds) ^a	24.56±7.67
9HPT ND (seconds) ^a	25.86±8.47
25WFT (seconds) ^a	6.75±2.6
SDMT ^a	40.59±13.47
BDI-II ^a	11.12±8.87
MFIS ^a	37.9±22.01
VAS-Qol ^a	68.91±21.22

Table 1. Demographic and clinical characteristics of PwMS a. Data are represented as means ± standard deviations. *ARR* annualized relapsing rate, *BDI-II* beck's depression inventory, *EDSS* expanded disability status scale, *MFIS* modified fatigue impact scale, *NA* not applicable, *PwMS* people with multiple sclerosis, *SDMT* symbol digit modality test, *VAS-Qol* EuroQol-5D visual analogue scale, *9HPT D* nine hole Peg test dominant hand, *9HPT ND* nine hole Peg test no dominant hand, *25WFT* 25-foot walking test.

8-OHdG), finding differences in pwMS compared to healthy controls, except for GR activity. Our results indicate that pwMS have an increase in the activation of certain antioxidant systems, such as SOD, while others, such as TAC and the levels of degradation products of oxidative stress 4-HNE and 8-OHdG, are lower than the control group. This is probably due to the increased oxidative stress in pwMS leading to excessive consumption of antioxidant components and, secondarily, a decrease in serum degradation products. In pwMS, TAC levels were associated with age, disease duration, and processing speed, while SOD correlated with T2 lesion load on MRI. Lower 4-HNE levels were linked to longer disease duration and increased processing speed, whereas 8-OHdG showed a correlation with the annual relapse rate (ARR).

Organisms have a series of defense mechanisms against pro-oxidant products, the most important of which are antioxidant enzymes²⁴. SOD is part of the physiological response to oxidative stress¹⁵ and its activity is increased in active demyelinating lesions in comparison to normal-appearing white matter and control groups^{14,25}. Our data, like those from previous studies, show increased SOD activity in pwMS^{26–28}. Not all results are homogenous, since other authors have observed decreased values in pwMS²⁹. In our case, higher SOD levels were associated with higher lesion burden, in contrast to Ljubicavljic et al. who found higher SOD activity in patients with a lower lesion load on T2. Furthermore, in their case they suggest that SOD could be negatively related to EDSS³⁰. The results of longitudinal studies are variable: some have observed a decrease in the relapse phase, followed by an increase³¹ Obradovic et al. found that SOD activity did not change after the relapse, but greater basal activity was associated with a better prognosis²⁷. As for the DMT, the effect of starting modifying drugs was studied, with the results being discordant^{32,33}.

Glutathione (GSH) is the most abundant antioxidant at the intracellular level and is fundamental to maintaining an appropriate cellular balance. Its metabolism is involved in the development of various neurodegenerative diseases³⁴. GR is a protein that triggers the regeneration of GSH. Prior studies are few and

	pwMS (n = 77)
Number of previous DMTs (mean) ^a	0.89±1.05
Exposure time to current DMT (months) ^a	64.86±67.23
DMT Efficacy	
Moderate n (%)	51 (66.2%)
High n (%)	23 (29.9%)
Without DMT n (%)	3 (3.9%)
MRI last year (46.7%, n = 36)	
T2 lesions	
≤9 n (%)	5 (13.88%)
10–20 n (%)	9 (25%)
>20 n (%)	22 (61.1%)
Gd+ lesions	4 (11%)
Vitamin D(ng/mL) ^a	22.41±11.71
Ferritin (ng/mL) ^a	75.44±86.56
Uric acid(mg/dL) ^a	4.27±1.83

Table 2. DMT, neuroimaging and laboratory characteristics of pwMS. ^a Data are represented as means ± standard deviations. *DMT* disease modifying treatment, *Gd* gadolinium, *MRI* magnetic resonance imaging, *NA* not applicable, *pwMS* people with multiple sclerosis.

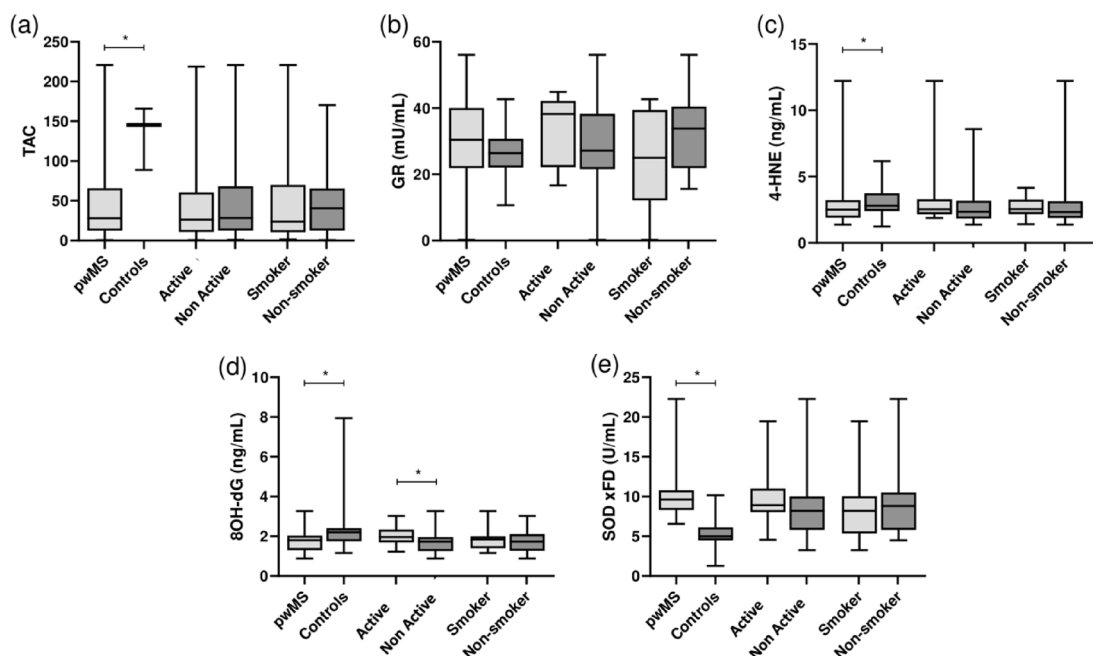


Fig. 1. Levels of antioxidant enzymes and degradation products of oxidative stress in pwMS and healthy controls, in pwMS smoker vs. non-smoker and pwMS with at least 1 relapse in the last year (active) vs. pwMS with no relapses (non-active). Levels of SOD (e) in pwMS compared to healthy controls, in which higher levels are observed in pwMS (a), (c) and (d) Levels of TAC, 4-HNE and 8-OHdG respectively in pwMS compared to healthy controls, whereby significantly lower levels are observed in pwMS (b) Levels of GR in pwMS compared to healthy controls. No differences have been found between smokers and non-smokers with multiple sclerosis (pwMS), nor between those with clinical activity versus those without relapses in the last year. Only in section d) were differences found in the levels of 8-OHdG, with higher levels in those with clinical activity in the last year compared to those without relapses. * Significant difference $p < 0.05$. *GR* glutathione reductase, *pwMS* people with multiple sclerosis, *SOD* superoxide dismutase, *TAC* total antioxidant capacity, *4-HNE* 4-Hydroxynonenal, *8-OHdG* 8-hydroxy 2 deoxyguanosine.

far between and show mixed results, partly due to the difference in the type of biochemical analysis. Jensen et al. measured the activity in erythrocytes, granulocytes and lymphocytes, observing a downward trend in pwMS that did not reach significance^{35,36} while other groups have found elevated activity in CSF and serum of pwMS^{37,38}. In our study, however we did not observe differences between the two groups.

	SOD (U/mL)	TAC	GR (mU/mL)	4-HNE (ng/mL)	8-OHdG (ng/mL)
Age					
R	0.234	0.243	-0.15	-0.19	-0.016
P-value	0.11	0.037*	0.23	0.16	0.224
Disease duration					
R	0.23	0.23	-0.20	-0.37	-0.11
P-value	0.11	0.047*	0.11	0.005*	0.38
EDSS					
R	0.02	0.22	0.12	-0.16	0.002
P-value	0.9	0.055	0.37	0.36	0.90
ARR					
R	0.154	-0.051	0.199	0.178	0.273
P-value	0.291	0.664	0.121	0.193	0.028*
MRI Gd + lesions					
R	-0.145	-0.119	0.336	0.099	0.247
P-value	0.530	0.496	0.060	0.608	0.160
DMT					
High efficacy ^a	9.59 ± 2.07	42.04 ± 47.55	32.09 ± 11.18	2.62 ± 1.56	1.77 ± 0.43
Moderate efficacy ^a	8.40 ± 4.03	50.85 ± 52.89	26.79 ± 13.08	3.05 ± 1.94	1.77 ± 0.57
P-Value	0.066	0.391	0.093	0.18	0.67
Fatigue					
Yes ^a	9.62 ± 4.59	55.06 ± 59.25	30.37 ± 12.96	2.6 ± 1.27	1.71 ± 0.42
No ^a	7.72 ± 2.00	48.19 ± 43.91	27.96 ± 11.88	3.34 ± 2.25	1.82 ± 0.64
P-Value	0.1402	0.9627	0.4136	0.1261	0.8311
Depression					
Yes ^a	9.30 ± 5.29	63.81 ± 62.82	28.09 ± 12.76	2.41 ± 0.49	1.69 ± 0.41
No ^a	8.17 ± 2.24	44.55 ± 45.53	29.35 ± 12.94	3.21 ± 2.14	1.80 ± 0.58
P-Value	0.99	0.1417	0.775	0.2355	0.694
SDMT					
≤38 ^a	9.42 ± 4.44	57.04 ± 53.74	29.24 ± 11.69	2.47 ± 0.89	1.69 ± 0.43
>38 ^a	7.89 ± 2.79	38.05 ± 46.50	28.84 ± 13.85	3.39 ± 2.33	1.81 ± 0.60
P-Value	0.148	0.072	0.885	0.045*	0.568

Table 3. Correlation between the parameters related to oxidative stress A.d different demographic, clinical A.d radiological variables. * Significant difference $p < 0.05$. A. Data A.e represented A. means ± standard deviations. ARR annualized relapsing rate, DMT disease modifying treatment, EDSS expanded disability status scale, gd gadolinium, GR glutathion reductase, MRI magnetic resonance imaging, SDMT symbol digit modality test, SOD Superoxido dismutase, TAC total A.tioxidant capacity, 4-HNE 4-Hydroxynonenal, 8-OHdG 8-hydroxy 2 deoxyguanosine.

Another way of measuring the antioxidant response is via the TAC, a parameter that incorporates not just the organism's intrinsic defense mechanisms, but also the antioxidant compounds we ingest as part of our diet³⁹. Its levels are modified by increased consumption in situations of greater oxidative stress or by increased intake of antioxidant foods. In our sample, TAC levels are lower in pwMS, consistent with other published papers^{37,40} in which lower TAC levels have actually been observed in patients with higher EDSS scores^{26,40–42}. Nonetheless, the results are heterogenous among studies⁴³ such as the study carried out by Khajenobar et al. in which observed higher TAC levels were observed in pwMS in both serum and CSF⁴⁴. We obtained higher values in pwMS with greater age and more time of evolution, without this being correlated with other parameters, such as EDSS. This could indicate lower consumption over time due to a less effective response to oxidative stress. This disparity in the findings may be influenced in part by diet and the consumption of lipoic acid, among other things. Diet can be a confusing factor that has not been measured in most studies, nor in ours⁴⁵.

Reactive species derived from oxidative stress generally have a very short half-life²⁴ so their detection in serum is very limited, making the analysis of products derived from oxidation of great interest. Specifically, oxidative damage to membrane lipids generates highly reactive aldehydes, including 4-HNE. This component has important effects in cell signaling, affecting transduction, differentiation and apoptosis. It plays a prominent role in neurodegenerative diseases and has been detected in the brain tissue of patients with Alzheimer's disease⁴⁶ MS¹⁴ and in CSF in the case of amyotrophic lateral sclerosis⁴⁷. This is the first study in which 4-HNE has been measured in the serum of pwMS, revealing significantly lower levels than in the control groups. Furthermore, we found greater levels of 4-HNE in patients with shorter disease duration, which could mean greater lipid degradation in the earlier stages of the disease. Another commonly used marker of nucleotide degradation is 8OH-dG, which is formed from the damage caused by hydroxyl radicals to deoxyguanosine. Studies of pwMS

		SOD (U/mL)	TAC	GR (mU/mL)	4-HNE (ng/mL)	8-OHdG (ng/mL)
Age	Estimate	0.22747	0.178281	-0.149717	-0.37647	-0.05039
	p-value	0.42011	0.55	0.5801	0.356	0.794
Disease duration	Estimate	-0.00355	0.204548	-0.2116351	-0.33764	-0.04564
	p-value	0.98617	0.409	0.3993	0.389	0.814
EDSS	Estimate	0.05258	0.159968	0.400186	-0.01534	-0.02054
	p-value	0.81567	0.514	0.050*	0.956	0.892
ARR	Estimate	0.22709	0.288105	0.117671	0.16992	0.09749
	p-value	0.27674	0.125	0.4176	0.419	0.368
MRI T2 lesions	Estimate	0.92112	0.115817	0.081274	-0.13217	-0.10095
	p-value	0.00173*	0.607	0.6446	0.605	0.454
Model parameters	Multiple R-squared	0.5863	0.2111	0.1838	0.2408	0.08052
	Adjusted R-squared	0.4484	0.05332	0.02679	0.07572	-0.08368
	F-statistic	4.251 on 5/15	1.338 on 5/25	1.171 on 5/26	1.459 on 5/23	0.4904 on 5/28
	Global p-value	0.01315*	0.2809	0.3501	0.2416	0.7806

Table 4. Multiple linear regression model for each oxidative stress variable in R. *ARR* annualized relapsing rate, *EDSS* expanded disability status scale, *GR* glutathione reductase, *MRI* magnetic resonance imaging, *SOD* Superoxido dismutase, *TAC* total antioxidant capacity, *4-HNE* 4-Hydroxynonenal, *8-OHdG* 8-hydroxy 2 deoxyguanosine. * Significant difference $p < 0.05$.

	With CI (SDMT ≤38)	Without CI (SDMT > 38)
Age (years) ^{a,*}	49.59 ± 8.67	38.82 ± 9.99
Disease duration (years) ^{a,*}	17.89 ± 9.89	10.91 ± 10.30
EDSS ^{a,*}	2.89 ± 1.51	1.37 ± 1.41
9HPT D (seconds) ^{a,*}	27.11 ± 9.66	22.11 ± 4.08
9HPT ND (seconds) ^{a,*}	28.79 ± 10.59	23.14 ± 4.62
25WFT (seconds) ^{a,*}	7.76 ± 2.97	5.78 ± 1.80
MFIS ^{a,*}	44.57 ± 18.14	31.22 ± 20.93
VAS-Qol ^{a,*}	59.85 ± 20.43	77.50 ± 18.42

Table 5. Mean values of variables with significant differences between PwMS with CI A.d without CI. A. Data A.e represented A. means ± standard deviations.* significant difference $p < 0,05$. *CI* cognitive impairment, *EDSS* expanded disability status scale, *MFIS* modified fatigue impact scale, *SDMT* symbol digit modality test, *VAS-Qol* EuroQol-5D visual A.alogue scale, *9HPT D* nine hole Peg test dominant hand, *9HPT ND* nine hole Peg test no dominant hand; *25WFT*: 25-foot walking test.

in which both are measured are scarce. We obtained lower levels of 8-OHdG in pwMS, which is an unexpected finding considering that previous neuropathological studies have reported elevated levels of 8OH-dG compared to normal-appearing white matter and control groups. However, in our study, we observed slightly higher levels in those pwMS who have experienced a relapse in recent months and is positively correlated with the ARR, which likely indicates an increase related to clinical activity. In other studies, serum determination of 8-OHdG has not produced homogeneous results, with both higher and³⁷ lower levels in pwMS⁴⁴. One possible explanation for the apparently contradictory findings of lower levels in the degradation products, both of 8-OHdG and of 4-HNE, could be that greater antioxidant activity in response to increased oxidative stress would produce a secondary decrease in peripheral levels of these degradation products¹⁴. In fact, Tasset et al. observed a decline in 8-OHdG after NTZ was started, suggesting that initiating DMT treatment is associated with increased activation of the antioxidant mechanisms³². Another interesting finding is the correlation between ferritin and 8-OHdG levels. Ferritin is a protein related to inflammation, neoplasia and infections. In previous studies, it was found to be related with MS, as well as oxidative stress biomarkers⁴⁸. The findings observed in our study suggest that ferritin may be associated with oxidative stress and DNA damage. However, we do not have ferritin levels in controls, so our results should be considered with caution.

The evaluation of symptoms associated with MS, such as CI, fatigue or depression, and their relationship with oxidative stress has been explored in very few articles, so our study provides new information on the role of oxidative stress and its potential as a biomarker of these symptoms. CI is very common among pwMS; it can be present from the early stages of the disease and affects up to 70% of patients. The most commonly affected areas are processing speed and learning and visual memory, although deficits may also appear in executive function, long-term memory or attention⁴⁹. Oxidative stress could play a role in the development of cognitive decline⁵⁰ and could even be a therapeutic target⁵¹but thus far, the studies in pwMS are scarce and have not yielded significant results. Naseri et al. assessed cognitive function using the MACFIMS battery and various

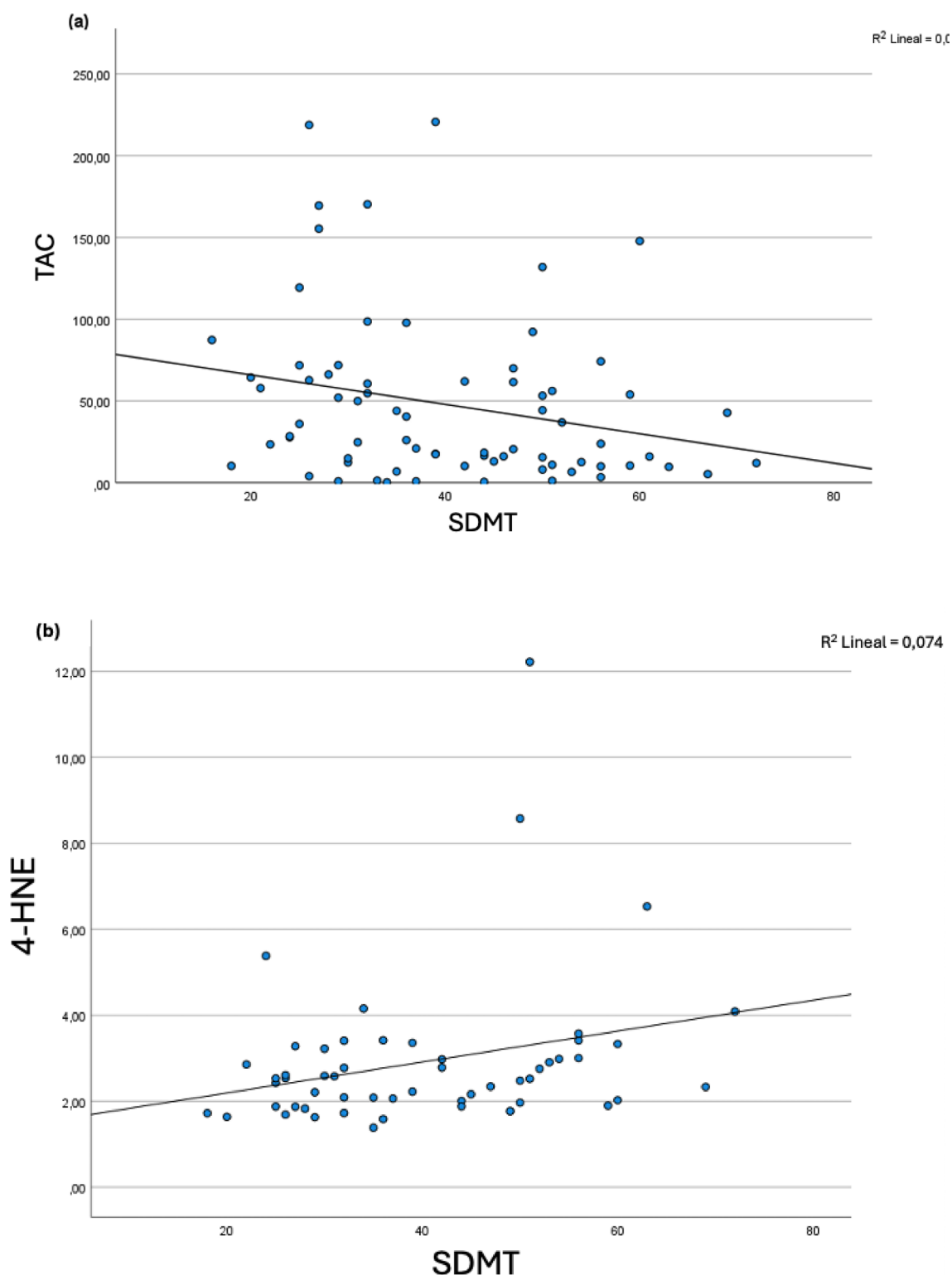


Fig. 2. Correlation between the SDMT score and TAC (a) and 4-HNE (b) levels, observing higher levels of 4-HNE and lower TAC levels in patients with higher SDMT scores. *SDMT* symbol digit modality test, *TAC* total antioxidant capacity, *4-HNE* 4-Hydroxynonenal.

parameters relating to oxidative stress, without finding differences except in glutathione peroxidase 1 levels⁵². In our study, we used a brief cognitive test, such as the SDMT, which fundamentally evaluates processing speed. The obtained results are of great interest, given that the levels of 4-HNE and TAC levels have been associated with the presence of CI and with a correlation with the test score. TAC levels are lower in pwMS with a higher score in the SDMT, which probably indicates increased oxidative stress in pwMS and CI, as well as increased compensatory

antioxidant activity and higher consumption of the antioxidant components. 4-HNE levels are lower in pwMS with lower SMDT scores, a finding consistent with the TAC data since increased antioxidant activity would lead to a decrease in degradation products. These results suggest that oxidative stress could play a contributing role in the development of cognitive problems. This may open new fields of research to improve our understanding how CI develops in MS and to identify biomarkers that correlate with symptoms of the disease. Moreover, if a specific treatment is found, its effect could be monitored. Further studies are required to evaluate its potential as a biomarker.

Depression is highly prevalent in MS, affecting 50% pwMS throughout their lifetime⁵³. An increase in oxidative stress parameters has been observed therein, and therapeutic trials have even been carried out with antioxidant agents for their treatment⁵⁴. Another of the most common symptoms in MS is fatigue, which is experienced by 80–90% of patients at any given time in their illness. This is defined as a lack of physical and/or mental energy perceived by the individual that interferes with their habitual activities^{55,56}. In the CNS, the presence of circulating proinflammatory cytokines promotes dysfunction in glutamate reuptake and a decrease in dopamine, both related to the development of fatigue and both capable of inducing an increase in oxidative stress through different pathways⁵⁷. Although MS, depression and fatigue share pathophysiological mechanisms associated with oxidative stress, the studies carried out thus far are scarce, with no differences have been found in fatigued or depressed patients^{33,58,59}, which is consistent with the observations of our study. Only Katarina et al. observed differences when analysing uric acid levels, finding a negative correlation with sadness on the BDI and with daily activities on the MFIS⁶⁰.

Until now, the link between stress and treatment with DMT whose use has been introduced in recent years, such as OCR, AZM or CLA, had not been evaluated. Despite the differences in terms of efficacy, no differences have been observed based on the DMT, even in patients being treated with DMF, to whom a special effect of oxidative stress is attributed via activation of the Nrf2 pathway⁶¹.

This study has various limitations. The small sample size has likely hindered the ability to obtain statistically significant results, making it necessary to conduct a more in-depth study with a larger number of pwMS. Patient selection was conducted in the MS clinic, where pwMS were offered the opportunity to participate. Selection bias may have occurred, as inclusion could have been influenced by the patient's level of cooperation. This is a cross-sectional study that does not enable us to draw inference about causality of the evolution of oxidative stress in relation to clinical, disease progression and radiological factors. Additionally, the study design does not allow for evaluating the impact of various DMTs on the levels of examined parameters. The use of commercial assay kits for evaluating oxidative stress markers in pwMS presents some inherent limitations that should be acknowledged. Firstly, while these kits offer convenience and standardized procedures, they are not diagnostic tools and are strictly for research purposes, potentially limiting their translational clinical applicability. Secondly, the specificity and sensitivity of such kits, may vary depending on the matrix (e.g., plasma, serum, urine) and require careful sample preparation to avoid pre-analytical variability. Matrix effects, such as interference from other biomolecules or hemolysis, can also skew results and complicate data interpretation. Additionally, the kits assume that the enzymatic reactions or immunoassays occur under ideal conditions, which may not.

fully replicate physiological environments or account for enzyme isoform variability relevant in MS pathophysiology. Finally, these kits provide snapshots rather than dynamic insights into oxidative stress processes, thus limiting conclusions about temporal fluctuations or causality in disease progression. It would also have been interesting to also know the levels of vitamin D, ferritin and uric acid in the control groups. The small number of pwMS receiving certain DMTs makes it very difficult to assess the relationship between oxidative stress parameters and these treatments. While the study considered some confounding factors such as age, sex or smoking, the influence of other parameters on oxidative stress such as physical activity and diet has not been explored. Although some findings have reached statistical significance, the magnitude of the observed correlation is weak, indicating that the relationship between the variables is limited in this context. This weakness may be due to the cross-sectional design of the study, which prevents the assessment of changes over time, as well as to the small sample size. Therefore, these results should be interpreted with caution and considered exploratory rather than definitive. Future studies with a longitudinal design and larger samples could help to better elucidate the relationship between these variables.

In conclusion, oxidative stress plays a prominent role not only in the pathophysiology of MS but also in other symptoms, such as fatigue or CI. Our results show an increase in antioxidant activity in pwMS that could have an influence on lower levels of products derived from it. Furthermore, the findings of our study suggest that some of these parameters may be related to clinical or radiological activity, although further studies are needed to confirm these results. The pwMS with slowing in processing speed have different oxidative stress profile, with higher TAC levels and lower values of lipid degradation products (4-HNE) being observed. Although it is not clear whether this relationship is causal in nature or simply reflects a consequence of other mechanisms our findings contribute to the understanding of the underlying pathophysiology. To date, no published studies have linked CI to oxidative stress factors. Our results could provide a basis for future research aimed at developing a biomarker associated with CI.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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References

- Compston, A. & Coles, A. Multiple sclerosis. *Lancet* **372**, 1502–1517. [https://doi.org/10.1016/S0140-6736\(08\)61620-7](https://doi.org/10.1016/S0140-6736(08)61620-7) (2008).
- Reich, D. S., Lucchinetti, C. F. & Calabresi, P. A. Multiple sclerosis. *N Engl. J. Med.* **378**, 169–180. <https://doi.org/10.1056/NEJMra1401483> (2018).
- Lassmann, H. & van Horssen, J. Oxidative stress and its impact on neurons and glia in multiple sclerosis lesions. *Biochim. Biophys. Acta* **1862**, 506–510. <https://doi.org/10.1016/j.bbadis.2015.09.018> (2016).
- Haider, L. et al. Oxidative damage in multiple sclerosis lesions. *Brain* **134**, 1914–1924. <https://doi.org/10.1093/brain/awr128> (2011).
- Alrouji, M. et al. Investigating the effect of cigarette smoking on serum uric acid levels in multiple sclerosis patients: A cross sectional study. *Brain Sci.* **13**. <https://doi.org/10.3390/brainsci13050800> (2023).
- Pegoretti, V. et al. Inflammation and oxidative stress in multiple sclerosis: consequences for therapy development. *Oxid. Med. Cell Longev.* 7191080 (2020). <https://doi.org/10.1155/2020/7191080>
- Centonze, D. et al. Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. *J. Neurosci.* **29**, 3442–3452. <https://doi.org/10.1523/JNEUROSCI.5804-08.2009> (2009).
- Campbell, G. R. & Mahad, D. J. Clonal expansion of mitochondrial DNA deletions and the progression of multiple sclerosis. *CNS Neurol. Disord. Drug Targets.* **11**, 589–597. <https://doi.org/10.2174/187152712801661194> (2012).
- Mahad, D. H., Trapp, B. D. & Lassmann, H. Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol.* **14**, 183–193. [https://doi.org/10.1016/S1474-4422\(14\)70256-X](https://doi.org/10.1016/S1474-4422(14)70256-X) (2015).
- Olesen, S. P. Free oxygen radicals decrease electrical resistance of microvascular endothelium in brain. *Acta Physiol. Scand.* **129**, 181–187. <https://doi.org/10.1111/j.1748-1716.1987.tb08057.x> (1987).
- Suneetha, A. & Raja Rajeswari, K. Role of dimethyl fumarate in oxidative stress of multiple sclerosis: A review. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **1019**, 15–20. <https://doi.org/10.1016/j.jchromb.2016.02.010> (2016).
- Mendiola, A. S. et al. Transcriptional profiling and therapeutic targeting of oxidative stress in neuroinflammation. *Nat. Immunol.* **21**, 513–524. <https://doi.org/10.1038/s41590-020-0654-0> (2020).
- Ohl, K., Tenbrock, K. & Kipp, M. Oxidative stress in multiple sclerosis: central and peripheral mode of action. *Exp. Neurol.* **277**, 58–67. <https://doi.org/10.1016/j.expneurol.2015.11.010> (2016).
- van Horssen, J. et al. Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. *Free Radic. Biol. Med.* **45**, 1729–1737. <https://doi.org/10.1016/j.freeradbiomed.2008.09.023> (2008).
- Ibitoye, R. et al. Oxidative stress-related biomarkers in multiple sclerosis: a review. *Biomark. Med.* **10**, 889–902. <https://doi.org/10.2217/bmm-2016-0097> (2016).
- Giovannoni, G. et al. Serum inflammatory markers and clinical/mri markers of disease progression in multiple sclerosis. *J. Neurol.* **248**, 487–495. <https://doi.org/10.1007/s004150170158> (2001).
- Borisovs, V., Bodrenko, J., Kalnina, J. & Sjakste, N. Nitrosative stress parameters and the level of oxidized DNA bases in patients with multiple sclerosis. *Metab. Brain Dis.* **36**, 1935–1941. <https://doi.org/10.1007/s11011-021-00786-5> (2021).
- Kalinowski, A. et al. The timed 25-foot walk in a large cohort of multiple sclerosis patients. *Mult Scler.* **28**, 289–299. <https://doi.org/10.1177/13524585211017013> (2022).
- Feys, P. et al. The Nine-Hole Peg test as a manual dexterity performance measure for multiple sclerosis. *Mult Scler.* **23**, 711–720. <https://doi.org/10.1177/1352458517690824> (2017).
- Flachenecker, P. et al. Fatigue in multiple sclerosis: a comparison of different rating scales and correlation to clinical parameters. *Mult Scler.* **8**, 523–526. <https://doi.org/10.1191/1352458502ms839oa> (2002).
- Beck, A. T., Mendelson, W. A. R. D. C. H., Mock, M., Erbaugh, J. & J. & An inventory for measuring depression. *Arch. Gen. Psychiatry.* **4**, 561–571. <https://doi.org/10.1001/archpsyc.1961.01710120031004> (1961).
- Group, E. EuroQol—a new facility for the measurement of health-related quality of life. *Health Policy.* **16**, 199–208. [https://doi.org/10.1016/0168-8510\(90\)90421-9](https://doi.org/10.1016/0168-8510(90)90421-9) (1990).
- Beier, M. et al. Proposed cut scores for tests of the brief international cognitive assessment of multiple sclerosis (BICAMS). *J. Neurol. Sci.* **381**, 110–116. <https://doi.org/10.1016/j.jns.2017.08.019> (2017).
- Kohen, R. & Nyska, A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol.* **30**, 620–650. <https://doi.org/10.1080/01926230290166724> (2002).
- Moezzi, D. et al. Expression of antioxidant enzymes in lesions of multiple sclerosis and its models. *Sci. Rep.* **12**, 12761. <https://doi.org/10.1038/s41598-022-16840-w> (2022).
- Acar, A. et al. Evaluation of serum oxidant/antioxidant balance in multiple sclerosis. *Acta Neurol. Belg.* **112**, 275–280. <https://doi.org/10.1007/s13760-012-0059-4> (2012).
- Obadovic, D., Andjelic, T., Ninkovic, M., Dejanovic, B. & Kotur-Stevuljevic, J. Superoxide dismutase (SOD), advanced oxidation protein products (AOPP), and disease-modifying treatment are related to better relapse recovery after corticosteroid treatment in multiple sclerosis. *Neurol. Sci.* **42**, 3241–3247. <https://doi.org/10.1007/s10072-020-04928-y> (2021).
- Tavassolifar, M. J. et al. Redox Imbalance in CD4+ T cells of relapsing-remitting multiple sclerosis patients. *Oxid Med Cell Longev.* 8860813 (2020). <https://doi.org/10.1155/2020/8860813>
- Rasoul, A. A. et al. The role of oxidative stress and haematological parameters in relapsing-remitting multiple sclerosis in Kurdish population. *Mult Scler. Relat. Disord.* **56**, 103228. <https://doi.org/10.1016/j.msard.2021.103228> (2021).
- Ljubisavljevic, S. et al. Cerebrospinal fluid and plasma oxidative stress biomarkers in different clinical phenotypes of neuroinflammatory acute attacks. Conceptual accession: from fundamental to clinic. *Cell. Mol. Neurobiol.* **33**, 767–777. <https://doi.org/10.1007/s10571-013-9944-5> (2013).
- Mitosek-Szewczyk, K., Gordon-Krajcer, W., Walendzik, P. & Stelmasiak, Z. Free radical peroxidation products in cerebrospinal fluid and serum of patients with multiple sclerosis after glucocorticoid therapy. *Folia Neuropathol.* **48**, 116–122 (2010).
- Tasset, I. et al. Effect of natalizumab on oxidative damage biomarkers in relapsing-remitting multiple sclerosis. *Pharmacol. Rep.* **65**, 624–631. [https://doi.org/10.1016/s1734-1140\(13\)71039-9](https://doi.org/10.1016/s1734-1140(13)71039-9) (2013).
- Adamczyk-Sowa, M. et al. Effect of melatonin supplementation on plasma lipid hydroperoxides, homocysteine concentration and chronic fatigue syndrome in multiple sclerosis patients treated with interferons-beta and Mitoxantrone. *J. Physiol. Pharmacol.* **67**, 235–242 (2016).
- Carvalho, A. N., Lim, J. L., Nijland, P. G., Witte, M. E. & Van Horssen, J. Glutathione in multiple sclerosis: more than just an antioxidant? *Mult Scler.* **20**, 1425–1431. <https://doi.org/10.1177/1352458514533400> (2014).
- Jensen, G. E., Gissel-Nielsen, G. & Clausen, J. Leucocyte glutathione peroxidase activity and selenium level in multiple sclerosis. *J. Neurol. Sci.* **48**, 61–67. [https://doi.org/10.1016/0022-510x\(80\)90150-1](https://doi.org/10.1016/0022-510x(80)90150-1) (1980).
- Jensen, G. E. & Clausen, J. Glutathione peroxidase and reductase, glucose-6-phosphate dehydrogenase and catalase activities in multiple sclerosis. *J. Neurol. Sci.* **63**, 45–53. [https://doi.org/10.1016/0022-510x\(84\)90107-2](https://doi.org/10.1016/0022-510x(84)90107-2) (1984).
- Tasset, I. et al. Peripheral oxidative stress in relapsing-remitting multiple sclerosis. *Clin. Biochem.* **45**, 440–444. <https://doi.org/10.1016/j.clinbiochem.2012.01.023> (2012).
- Calabrese, V., Raffaele, R., Cosentino, E. & Rizza, V. Changes in cerebrospinal fluid levels of malondialdehyde and glutathione reductase activity in multiple sclerosis. *Int. J. Clin. Pharmacol. Res.* **14**, 119–123 (1994).
- Apak, R. et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* **12**, 1496–1547. <https://doi.org/10.3390/12071496> (2007).

40. Besler, H. T. & Comoğlu, S. Lipoprotein oxidation, plasma total antioxidant capacity and homocysteine level in patients with multiple sclerosis. *Nutr. Neurosci.* **6**, 189–196. <https://doi.org/10.1080/1028415031000115945> (2003).
41. Armon-Omer, A. et al. New insights on the nutrition status and antioxidant capacity in multiple sclerosis patients. *Nutrients* **11** <https://doi.org/10.3390/nu11020427> (2019).
42. Hadžović-Džuvo, A. et al. Serum total antioxidant capacity in patients with multiple sclerosis. *Bosn J. Basic. Med. Sci.* **11**, 33–36. <https://doi.org/10.17305/bjbms.2011.2620> (2011).
43. Adamczyk, B., Wawrzyniak, S., Kasperczyk, S. & Adamczyk-Sowa, M. The evaluation of oxidative stress parameters in serum patients with relapsing-remitting multiple sclerosis treated with II-Line Immunomodulatory therapy. *Oxid. Med. Cell. Longev.* **2017** (9625806). <https://doi.org/10.1155/2017/9625806> (2017).
44. Khajenobar, N. B. et al. Comparison between cerebrospinal fluid and serum levels of myelin-associated glycoprotein, total antioxidant capacity, and 8-hydroxy-2'-deoxyguanosine in patients with multiple sclerosis. *Clin. Neurol. Neurosurg.* **200**, 106377. <https://doi.org/10.1016/j.clineuro.2020.106377> (2021).
45. Khalili, M. et al. Effect of lipoic acid consumption on oxidative stress among multiple sclerosis patients: a randomized controlled clinical trial. *Nutr. Neurosci.* **17**, 16–20. <https://doi.org/10.1179/1476830513Y.00000000060> (2014).
46. Benedetti, E. et al. Involvement of peroxisome proliferator-activated receptor β/δ (PPAR β/δ) in BDNF signaling during aging and in Alzheimer disease: possible role of 4-hydroxynonenal (4-HNE). *Cell. Cycle* **13**, 1335–1344. <https://doi.org/10.4161/cc.28295> (2014).
47. Smith, R. G., Henry, Y. K., Mattson, M. P. & Appel, S. H. Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. *Ann. Neurol.* **44**, 696–699. <https://doi.org/10.1002/ana.410440419> (1998).
48. Ferreira, K. P. Z. et al. Disease progression and oxidative stress are associated with higher serum ferritin levels in patients with multiple sclerosis. *J. Neurol. Sci.* **373**, 236–241. <https://doi.org/10.1016/j.jns.2016.12.039> (2017).
49. Chiaravalloti, N. D. & DeLuca, J. Cognitive impairment in multiple sclerosis. *Lancet Neurol.* **7**, 1139–1151. [https://doi.org/10.1016/S1474-4422\(08\)70259-X](https://doi.org/10.1016/S1474-4422(08)70259-X) (2008).
50. Hajjar, I. et al. Oxidative stress predicts cognitive decline with aging in healthy adults: an observational study. *J. Neuroinflammation* **15**, 17. <https://doi.org/10.1186/s12974-017-1026-z> (2018).
51. Tarbali, S. & Khezri, S. Vitamin D3 attenuates oxidative stress and cognitive deficits in a model of toxic demyelination. *Iran. J. Basic. Med. Sci.* **19**, 80–88 (2016).
52. Naseri, A. et al. Circulatory antioxidant and oxidative stress markers are in correlation with demographics but not cognitive functions in multiple sclerosis patients. *Mult Scler. Relat. Disord.* **57**, 103432. <https://doi.org/10.1016/j.msard.2021.103432> (2022).
53. Feinstein, A. The neuropsychiatry of multiple sclerosis. *Can. J. Psychiatry* **49**, 157–163. <https://doi.org/10.1177/070674370404900302> (2004).
54. Morris, G. et al. Multiple Immune-Inflammatory and oxidative and nitrosative stress pathways explain the frequent presence of depression in multiple sclerosis. *Mol. Neurobiol.* **55**, 6282–6306. <https://doi.org/10.1007/s12035-017-0843-5> (2018).
55. Giovannoni, G. Multiple sclerosis related fatigue. *J. Neurol. Neurosurg. Psychiatry* **77**, 2–3. <https://doi.org/10.1136/jnnp.2005.074948> (2006).
56. Krupp, L. Fatigue is intrinsic to multiple sclerosis (MS) and is the most commonly reported symptom of the disease. *Mult Scler.* **12**, 367–368. <https://doi.org/10.1191/135248506ms1373ed> (2006).
57. Morris, G., Berk, M., Galecki, P., Walder, K. & Maes, M. The Neuro-Immune pathophysiology of central and peripheral fatigue in systemic Immune-Inflammatory and Neuro-Immune diseases. *Mol. Neurobiol.* **53**, 1195–1219. <https://doi.org/10.1007/s12035-015-9090-9> (2016).
58. Pasquali, L. et al. Plasmatic oxidative stress biomarkers in multiple sclerosis: relation with clinical and demographic characteristics. *Clin. Biochem.* **48**, 19–23. <https://doi.org/10.1016/j.clinbiochem.2014.09.024> (2015).
59. Krysko, K. M. et al. A pilot study of oxidative pathways in MS fatigue: randomized trial of N-acetyl cysteine. *Ann. Clin. Transl. Neurol.* **8**, 811–824. <https://doi.org/10.1002/acn3.51325> (2021).
60. Katarina, V., Gordana, T., Svetlana, M. D. & Milica, B. Oxidative stress and neuroinflammation should be both considered in the occurrence of fatigue and depression in multiple sclerosis. *Acta Neurol. Belg.* **120**, 853–861. <https://doi.org/10.1007/s13760-018-1015-8> (2020).
61. Linker, R. A. et al. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain* **134**, 678–692. <https://doi.org/10.1093/brain/awq386> (2011).

Author contributions

All authors contributed to the study conception and design. R.P.M.: Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing - Original Draft, Review & Editing, Visualization, Supervision, Project administration. R.D.O.: Methodology, Resources, Investigation, Writing - Review & Editing. F. J. B. H.: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - Review & Editing, Visualization, Supervision. M.J.G.: Resources, Investigation. A.B.: Resources, Investigation. P.A.G.: Formal analysis. F.V.M.: Supervision.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to R.P.-M.

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