

Oxidative stress in patients with newly diagnosed multiple sclerosis: any association with subclinical atherosclerosis?

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Abstract

OBJECTIVES: Multiple sclerosis (MS) is a chronic inflammatory autoimmune and neurodegenerative disease of the central nervous system (CNS) typically affecting young adults. Although the pathogenesis of MS is not fully understood, there is evidence to suggest that inflammation-induced oxidative stress can play a role in demyelination and axonal damage. Oxidative stress also participates in the pathogenesis of endothelial dysfunction and atherogenesis. Data from large epidemiological studies showed a higher risk of vascular events in MS patients. The aim of our study was to analyse the presence of oxidative stress and its association with the parameters of subclinical atherosclerosis in the early stages of MS.

MATERIAL AND METHODS: We compared 13 newly diagnosed MS patients with a group of 13 healthy age- and BMI-matched controls. Blood samples were measured for total antioxidant activity using TEAC assay. Endothelial function, expressed as reperfusion hyperaemia index (RHI) and arterial stiffness, expressed as augmentation index standardized to a pulse of 75/min (AI@75) were assessed using peripheral arterial tonometry.

RESULTS: MS patients had significantly lower TEAC compared to controls [0.8 (0.4-2.4) vs. 1.2 (0.6-3.8) mmol/l; $p=0.004$]. The frequency of increased arterial stiffness (61.6% vs. 30.8%) and endothelial dysfunction (46.2% vs. 38.5%) was comparable in MS patients and in controls. There was no significant association between TEAC, increased arterial stiffness or endothelial dysfunction in patients and controls.

CONCLUSION: Our study showed decreased antioxidant capacity in newly diagnosed MS patients compared to controls. We failed to find association of subclinical atherosclerosis with oxidative stress in newly diagnosed MS.

Abbreviations:

AI	- augmentation index
AI@75	- augmentation index standardized to a pulse of 75/min
BMI	- body mass index
CNS	- central nervous system
ED	- endothelial dysfunction
EDSS	- Expanded Disability Status Scale
IQR	- interquartile range
MS	- multiple sclerosis
RHI	- reperfusion hyperaemia index
RNS	- reactive nitrogen species
ROS	- reactive oxygen species
TAC	- total antioxidant capacity
TEAC	- Trolox equivalent antioxidant capacity
TG	- triglyceride

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) which typically affects young adults in working age and is one of the most common causes of invalidity (Compston & Coles, 2008; Trapp & Nave, 2008). The etiology of MS is still not completely understood (Ebers, 2008; Durfinova *et al.* 2014). Genetic, environmental and other factors may contribute to triggering an aberrant autoimmune attack which may result in the damage of the myelin and axons (Stadelmann & Bru, 2005).

Oxidative stress is a condition with a characteristic imbalance between concentrations of reactive oxygen species (ROS) and antioxidants. Some ROS are extremely unstable and have strong oxidation activity. Excessive ROS production leads to cellular injury, such as damage to DNA, proteins, carbohydrates, and lipid membranes (Preiser, 2012). The cellular damage caused by ROS has been implicated in the development of many disease states such as cancer, diabetes, vascular disease, atherosclerosis, and neurodegenerative diseases (Liguori *et al.* 2018).

It's believed that oxidative stress also contributes to the pathogenesis of MS (Bartova *et al.* 2016). Activated macrophages and astrocytes can produce large amounts of ROS or reactive nitrogen species (RNS) (Croxford *et al.* 2015). Oxygen consumption in the CNS is high, and the cell membranes are rich in polyunsaturated fatty acids which are susceptible to peroxidation. In MS patients, the concentration of antioxidant substances in the CNS was lower than that in peripheral blood, so their consumption during oxidative stress was suggested (Garg & Smith, 2015; Ohl *et al.* 2016). Based on the above data, neural cells – compared to other body tissues - are more susceptible to oxidative damage.

Oxidative stress can play an important role in the pathogenesis of atherosclerosis, and the uncontrolled production of ROS is implicated in the vascular injury (Kattoor *et al.* 2017). Endothelial dysfunction (ED) is an early predictor of vascular disease and it is considered to be a subclinical stage of atherosclerosis. It's commonly described as the inability of the artery to suffi-

ciently dilate in response to an appropriate endothelial stimulus (Vanhoutte *et al.* 2017; Daiber *et al.* 2017). ED could be involved in the pathogenesis of MS. Dysfunction of cerebral endothelial cells causes adherence and trans-endothelial migration of T-lymphocytes and monocytes to the central nervous system (Christiansen, 2012).

In epidemiological studies, the higher risk of vascular events such as acute coronary syndrome and stroke was found in MS subjects (Thormann *et al.* 2016). Both oxidative stress and ED are supposed to be involved in the pathogenesis of MS. Oxidative stress is also a risk factor for developing ED. The aim of our study was to compare the parameters of oxidative stress in MS patients and in matched healthy controls and to explore the association of oxidative stress markers with parameters of subclinical atherosclerosis.

MATERIAL & METHODSSubjects

We enrolled 13 patients with newly-diagnosed yet untreated MS (mean age 29.8±6.6 years, body mass index [BMI] 23.0±2.3) and age, sex and BMI-matched population of 13 healthy volunteers with a low burden of vascular risk factors (mean age 30.8±6.9 years, BMI 23.0±2.7). The MS patients were recruited from the registry of patients hospitalized at the 1st Department of Neurology, Medical Faculty, Comenius University Bratislava. All MS patients met the McDonalds' criteria for diagnosis of MS, and only patients with newly diagnosed MS were included (Polman *et al.* 2011). Individuals participating in our study signed an informed consent. This study was approved by the local ethics committee.

Exclusion criteria for both groups included: any internal diseases, current use of intravenous corticosteroids, chronic medication and smoking. All clinical characteristics were scored at the time of endothelial function assessment and blood sampling. The inclusion criterion for MS patients was also Expanded Disability Status Scale (EDSS) ≤ 2. The patients were asked to fast for 12 hours before the examination and to avoid any excessive physical activity 12 hours before the examination.

Assessment of antioxidant capacity

All patients' and controls' blood samples were obtained after overnight fasting. Collected blood was centrifuged for 5 min at 1200 × g, at 4°C and the serum samples were stored in aliquots at -70 °C for the determination of total antioxidant capacity (TAC). Assessment was established by the Trolox equivalent antioxidant capacity (TEAC) assay (Re *et al.* 1999). This measures the ability of antioxidants to scavenge the stable radical cation ABTS^{•+} (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), a blue-green chromophore with maximum absorption at 734 nm that decreases its intensity

Tab. 1. Characteristics of the study groups

	MS patients	Controls	P
N	13	13	
Age (years)	29.8±6.6	30.8±6.9	0.954
Females/males	7/6 (53.8/46.2%)	6/7 (46.2/53.8%)	0.695
Body-mass index (kg/m ²)	23.0±2.3	23.0±2.7	0.991
Systolic BP (mmHg)	116.2±12.4	120.4±14.6	0.442
Diastolic BP (mmHg)	76.8±10.5	74.8±7.5	0.567
T-chol (mmol/l)	4.3±0.5	4.0±0.6	0.311
LDL cholesterol (mmol/l)	2.8±0.4	2.5±0.5	0.106
HDL cholesterol (mmol/l)	1.3±0.3	1.3±0.3	0.648
TG (mmol/l)	0.3±0.1	0.4±0.2	0.598
RHI	2.0±0.8	2.0±0.6	0.947
Endothelial dysfunction (RHI≤1.67)	6 (46.2%)	5 (38.5%)	1.000
AI@75	-6.0; 12.5 (-28.0-12.0)	-12.0; 24.5 (-33.0-61.0)	0.336
Increased arterial stiffness (AI@75>-10)	8/13 (61.6%)	4/13 (30.8%)	0.237
TEAC (mmol/l)	0.8; 0.4 (0.4-2.4)	1.2; 2.1 (0.6-3.8)	0.004***

Values in table are presented as n (%), mean ± standard deviation or median, interquartile range (min–max). ****p* < 0.001. BP: blood pressure, T-chol: total cholesterol, TG: triglycerides, RHI: reperfusion hyperaemia index, AI@75: arterial stiffness normalized to heart rate of 75 beats per minute, TEAC: Trolox equivalent antioxidant capacity.

in the presence of antioxidants. Antioxidants can neutralize the radical cation ABTS⁺, generated from ABTS, by either direct reduction via electron donation or by the radical quenching via hydrogen atom donation, and the balance of these two mechanisms is generally determined by the antioxidant structure and pH of the medium (Zhong & Shahidi, 2015).

Lipid profile measurement

Fasting serum total cholesterol (T-chol), LDL cholesterol, HDL cholesterol, and triglyceride (TG) levels were determined using an autoanalyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) by standard procedures with enzymatic kits (Roche Diagnostics, Lewes, UK).

Assessment of endothelial function

Assessment of endothelial function was performed non-invasively using the EndoPAT 2000 device (Itamar Medical, Caesarea, Israel). The measurement was calculated using a computerized automated algorithm (software version 3.1.2) provided with the device. We calculated reperfusion hyperaemia index (RHI), which is a parameter of endothelial function, and augmentation index (AI), which is a parameter of arterial stiffness. AI@75 is AI standardized to a pulse of 75/min (AI@75).

Measurements were performed according to the manufacturer's instructions as previously described. The subjects were asked to remain still and silent during the entire measurement period. Each record-

ing consisted of 5 minutes of baseline measurement, 5 minutes of occlusion measurement and 5 minutes of post-occlusion measurement (hyperaemic period). Occlusion of the brachial artery was performed on the non-dominant upper arm. The occlusion pressure was at least 60 mmHg above the systolic blood pressure. The RHI measure of ≤1.67 was considered as ED (Moerland et al. 2012; Siarnik et al. 2014).

Statistical Analysis

The analyses were assessed with SPSS version 18 (SPSS Inc., Chicago, USA). Categorical variables were expressed as numbers (%), continuous variables as means (± standard deviation) or median (interquartile range [IQR], minimal-maximal values).

The Fisher Exact Test, Mann-Whitney U test, and Student t test were used for group comparison of particular variables between MS patients and controls. The Spearman rank correlation was used to determine the relationships between RHI and TEAC. All tests were 2-sided and values of *p* < 0.05 were considered statistically significant.

RESULTS

Characteristics of the study group are included in Table 1. All baseline anthropometric parameters were comparable in MS patients and healthy controls. There was no significant difference in fasting serum concentration either in total, LDL or HDL cholesterol, nor TG between MS patients and healthy controls (Table 1). In

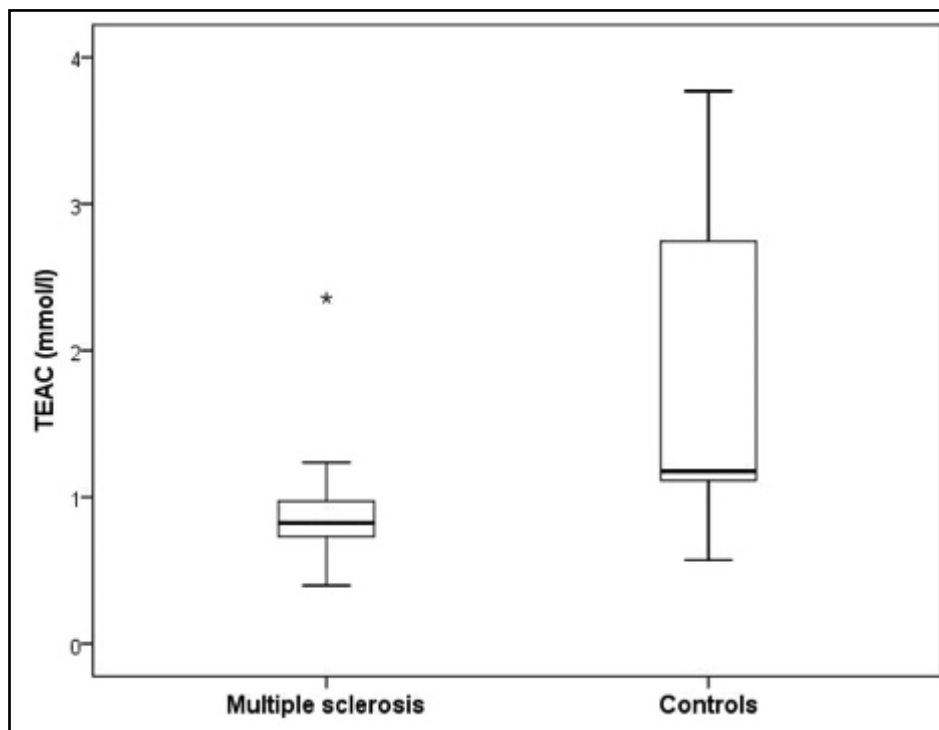


Fig. 1. Values of TEAC in MS patients compared to controls (median 0.8, range: 0.4-2.4 vs. median 1.2, range: 0.6-3.8, $p=0.004$). Asterisk represent extreme values of TEAC.

MS patients, compared to controls, significantly lower values of TEAC were found [0.8 (0.4-2.4) vs. 1.2 (0.6-3.8), $p=0.004$].

We failed to find any significant correlation between TEAC and RHI ($r=0.011$; $p=0.972$), as well as between TEAC and AI@75 ($r=0.077$, $p=0.789$) in MS group. There was no significant correlation between TEAC and RHI ($r=0.093$, $p=0.762$) as well as between TEAC and AI@75 ($r=-0.376$, $p=0.206$) in controls. The frequency of increased arterial stiffness and ED was comparable in MS patients and controls (Table 1).

DISCUSSION

The main finding of this study is the observation of significantly lower serum antioxidant capacity in MS patients compared to the healthy control group. The lower antioxidant protection could lead to the higher oxidative injury of the CNS. The frequency of increased arterial stiffness and ED was comparable in MS patients and controls. We failed to find any significant associations between subclinical atherosclerosis measures and the TEAC values.

The pathogenesis of MS is characterized by a cascade of pathophysiological events beginning with a focal infiltration of the CNS lymphocytes and activation of microglia, resulting in demyelination and axonal degeneration (Patergnani *et al.* 2017). The development of neurodegeneration in MS is a complex process that may be related to primary apoptosis, synaptopathy, excitotoxicity associated with glutamate overload, ionic

channel dysfunction, calcium overload, mitochondriopathy, proteolytic enzyme production and activation of apoptotic pathways. It is also important that mitochondrial dysfunction results in an increased production of ROS which is detrimental to neurons and glia (Adamczyk & Adamczyk-Sowa, 2016; Errea *et al.* 2015). Alternatively, MS could be primarily a degenerative disease where the oxidative stress and mitochondrial dysfunction could play a crucial role (Horsen *et al.* 2017). Our results support the hypothesis that oxidative stress plays an important role in MS pathogenesis, however, we are not able to specify the causality.

The results of multiple population-based studies suggest that patient with MS have a higher risk of vascular events. Patients with MS were reported to have an approximate 30% higher risk of cardiovascular mortality than the age-matched general population (Khan, 2010; Koch-Henriksen *et al.* 1998). Thormann *et al.* (2016) conducted a combined case-control and cohort study where all Danish born citizens with the onset of MS between 1980-2005 were identified from the Danish Multiple Sclerosis Registry and randomly matched with controls. MS cases had an increased risk for cerebrovascular comorbidity [hazard ratio 1.84 (95% CI 1.69-2.00, $p < 0.0005$)] and for cardiovascular comorbidity [hazard ratio 1.08 (95% CI 1.02-1.15, $p = 0.013$)]. Marrie *et al.* (2019) found an increased incidence of acute myocardial infarction in MS population independent of traditional vascular risk factors. They identified 14,565 persons with MS and 72,825 matched controls. After adjustment, the risk of acute myocardial

infarction was 60% higher in the MS population than in the matched control population (hazard ratio 1.63; 95% CI 1.43–1.87).

The increased risk of vascular disease in MS might be caused by the ED which is believed to be the most important initial step in the process of atherogenesis. Kemenyova *et al.* (2015) proved significant impairment of endothelial function in MS population compared to age matched control population with low burden of vascular risk factors. On the contrary, in our study we have not found any statistically significant differences in endothelial function (assessed as RHI) or in arterial stiffness (assessed as AI@75) in MS patients compared to the control group. This difference could be caused by different spectrum of enrolled patients. Our patients were newly diagnosed and without any chronic treatment, so the endothelial function was not affected by corticosteroid pulse, use of disease-modifying therapies, nor by the duration of the disease. Patients in our study had low disability score (EDSS \leq 2) and had an active lifestyle before the diagnosis was set up.

In our study, we failed to find any significant associations between measures of subclinical atherosclerosis and the values of TEAC. Atherogenesis, including ED, is associated with multiple traditional vascular risk factors such as dyslipidaemia, arterial hypertension, hyperglycaemia and diabetes. Despite including only previously healthy subjects to our study, we were not able to avoid all potential vascular risk factors in this population. For example, Penesova *et al.* (2015) proved impaired glucose metabolism and impaired insulin action compensated by hyperinsulinaemia in newly diagnosed, previously untreated patients with MS. Similarly, dyslipidaemia and impairment of autonomic dysfunction was found in previously healthy patients with newly diagnosed MS (Radikova *et al.* 2018; Vlcek *et al.* 2018). Despite the fact that oxidative stress is considered an important mechanism in atherogenesis, we failed to find any significant associations between the measures of subclinical atherosclerosis and the values of TEAC in both groups. This finding might suggest that oxidative stress does not seem to be a key player in atherogenesis in newly diagnosed MS patients and there is the possibility of other more important atherogenic factors in MS patients. On the other hand, we suggest that atherogenic implications of decreased antioxidant capacity in our MS population could be blunted by the short duration of the disease, and the atherogenic consequences of the oxidative stress may become more evident in patients with a longer duration of the disease. The main limitations of our study are a small sample size and the absence of detailed screening for traditional and non-traditional atherogenic risk factors (for example autonomic dysfunction, physical activity, impairment of glucose metabolism); these should be included in future prospective longitudinal studies to elucidate predictors of atherogenesis in MS patients.

CONFLICT OF INTEREST

None.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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