Serum total antioxidant capacity in patients with multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). It is characterized by loss of myelin, the fatty tissue that surrounds and protects nerve fibres allowing them to conduct electrical impulses. Recent data indicate that oxidative stress (OS) plays a major role in the pathogenesis of multiple sclerosis (MS). The aim of this study was to estimate level of serum total antioxidative capacity in patients with multiple sclerosis. Our cross-sectional study included 33 patients with MS and 24 age and sex matched control subjects. All our patients had a Poser criteria for definite diagnostic categories of multiple sclerosis. Serum total antioxidant capacity (TAC) was measured by quantitative colorimetric determination, using Total antioxidant Capacity-QuantiCromAntioxidant Assay Kit (BioAssay systems, USA; DTAC-100). Mean serum TAC in multiple sclerosis group of patients was 119.2 mM Trolox equivalents and was significantly lower (p<0.001) compared to the control group of subjects (167.1 mM Trolox equivalents). Our results showed that oxidative stress plays an important role in pathogenesis of multiple sclerosis. This finding, also, suggests the importance of antioxidants in diet and therapy of MS patients.

KEY WORDS: multiple sclerosis, total antioxidant capacity (TAC), oxidative stress

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). It is characterized by loss of myelin, the fatty tissue that surrounds and protects nerve fibres allowing them to conduct electrical impulses. Macroscopic examination of CNS tissue of individuals with MS reveals multiple sharply demarcated plaques in the white matter with destruction of myelin sheaths, oligodendrocyte damage and cell death, axonal damage and axonal loss, glial scar formation and the presence of inflammatory infiltrates that mainly consist of lymphocytes and macrophages [1]. The cause of MS is unknown. According to the results of previous studies particular monocyte-derived macrophages are thought to play a central role in MS pathology, although others speculate that MS is a vascular disease. As macrophages produce various mediators (cytokines, nitric oxide, reactive oxygen species), there are a lot of factors that could contribute to pathogenesis and development of MS [2]. In addition recent data indicate that oxidative stress (OS) plays a major role in the pathogenesis of multiple sclerosis [2]. The exact mechanisms responsible for increased oxidative stress in MS patients needs to be further explored and there is not yet enough evidence to decide whether oxidative stress is a primary phenomenon inducing the progression of MS. Free radicals are common outcome of normal aerobic cellular metabolism. In-built antioxidant system of body plays its decisive role in prevention of any loss due to free radicals. Imbalanced defence mechanism of antioxidants, caused by overproduction of free radicals or their incorporation from environment to living system, leads to damage and neuro-degeneration, subsequently. Neural cells suffer functional or sensory loss in neurodegenerative diseases. Apart from several other environmental or genetic factors, oxidative stress (OS) leading to free radical attack on neural cells contributes calamitous role to neuro-degeneration. Though, oxygen is imperative for life, imbalanced metabolism and excess reactive oxygen species (ROS) generation end into a range of disorders such as MS [3]. Reactive oxygen species (ROS), leading to OS, generated in excess primarily by macrophages, have been implicated as mediators of demyelization and axonal damage in both MS and experimental autoimmune encephalomyelitis (EAE), its animal model [4]. ROS cause damage at the level of cellular components such as lipids, proteins and nucleic ac-

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ids (e. g., RNA, DNA), resulting in cell death by necrosis or apoptosis. In addition, weakened cellular antioxidant defence systems in the central nervous system (CNS) in MS, and its vulnerability to ROS effects may increase damage [4]. Treatment with antioxidants might theoretically prevent propagation of tissue damage and improve both survival and neurological outcome of the disease [4]. One of the parameters that is modulated either by radical overload or by intake of dietary antioxidants (and can therefore be regarded as more representative of the in vivo balance between oxidizing species and antioxidant compounds unknown, measurable and not measurable) is plasma total antioxidant capacity (TAC). Studies related to the assessment of total antioxidant capacity/TAC and its diagnostic significance in body fluids are lacking and deserve further attention [5]. The aim of this study was to estimate level of serum total antioxidative capacity in patients with multiple sclerosis.

Materials and Methods

Subjects

This study was approved by the Research Ethical Committee of the Clinical Centre University of Sarajevo and conducted at Department of Neurology, Clinical Centre University of Sarajevo. In total, 33 (37.9 ± 1.3 years) randomly selected patients with various types of multiple sclerosis and 24 apparently healthy sex- and age-matched person (38.7 ± 1.1 years), serving as control group, were tested. Informed consent according to the declaration of Helsinki was obtained from each subject at the time of recruitment to the study. The patients and healthy controls had received neither steroid therapy nor vitamin supplementation, and were non-smokers. All our patients fulfilled Poser criteria [6] for definite diagnostic categories for multiple sclerosis. For both groups of subjects, exclusion criteria were signs of mental deterioration (MMSE score < 26), EDSS scores >7, as well as patients with signs of acute somatic or other neurological diseases. Disability was graded using the Kurtzke Expanded Disability Status Scale (EDSS) [7]. It is an ordinal scale with 19 disease steps between 0 and 10, whereby a higher score indicates greater disability. Global cognitive function was tested with the 30-point MMSE score [8]. Further data were collected on the type of disease, the duration and the presence of symptoms, and sociodemographic characteristics of subjects. Data were obtained from the anamnesis and by using the history of the disease.

Assessment of serum Total Antioxidant Capacity

Analysis of TAC was performed at Department of Biochemistry, Faculty of Medicine Sarajevo. From each subject 5 ml of blood sample were taken from the antecubital vein, in fasting state. Blood samples were centrifuged at 4°C for 10 min at 2500 rpm to obtain serum. The serum samples were stored at -80°C until analysis. The samples were thawed at room temperature only once at the time of assay. Serum total antioxidant capacity was measured by quantitative colorimetric assay, using Total antioxidant Capacity - QuantiCromAntioxidant Assay Kit (BioAssay systems, USA; DTAC-100). By this method Cu^{2+} is reduced by antioxidant to Cu^{+}. The resulting Cu^{+} specifically forms a colored complex with a dye reagent. The colour intensity at 570 nm is proportional to TAC in the simple. Serum total antioxidant capacity is expressed in mM Trolox equivalents. Referral TAC values using this kit is within the linear range of 1.5 – 1000 mM Trolox equivalents.

Statistical Analysis

Data distribution was determined using the Kolmogorov-Smirnov test and presented as mean ± SEM. Difference in TAC values between the two groups were tested with the non-parametric Mann–Whitney U test. Two-tailed p values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS statistical software system (version 16.0, SPSS Inc, Chicago, Illinois, USA).

Results

Mean serum TAC in multiple sclerosis group of patients was 119.2 mM Trolox equivalents and was significantly lower (p<0.001) compared to the control group of subjects (167.1 mM Trolox equivalents) (Figure 1).

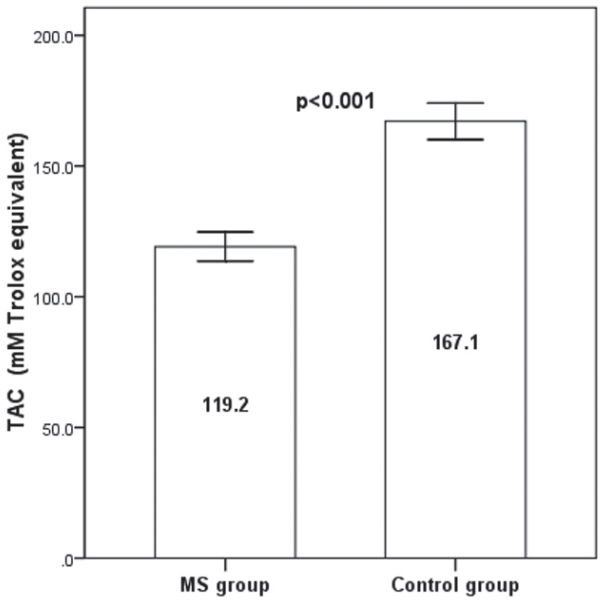

FIGURE 1. Mean serum total antioxidative capacity concentration in MS group and control group. Bars show means and error bars show ±SEM; MS group - multiple sclerosis group

Discussion

Perturbation of the cellular oxidant/antioxidant balance (oxidative stress) has been suggested to be involved in the neuropathogenesis of several disease states, including stroke, MS, Parkinson disease, Alzheimer's disease, as well as "normal" physiological aging [9,10]. The possible links between MS and perturbation of oxidant/ antioxidant cell balance may be supported by several factors including low intake of vitamin E, increased malonaldehyde levels in blood and decreased glutathione peroxidase activity in erythrocytes, lymphocytes, and granulocytes of patients with multiple sclerosis, increased amounts of lipid peroxidation products in both their plasma and CSF, and, in addition, an inappropriate expression of heat shock proteins on oligodendrocytes [11]. It is a well known fact that under normal circumstances, the potential damaging effects of these free radicals are limited by the endogenous antioxidant defences in body [11]. Decreased glutathione and alpha tocopherol concentrations, normal ascorbic acid and increased uric acid levels observed in demyelinating plaques [4] are fully supportive of this statement. Dietary antioxidants such as vitamins appear to be of great importance for the control of the effects of reactive oxygen species. Retinol, beta carotene, alpha tocopherol and ascorbic acid are significantly lower in MS patients compared to controls [12]. Although vitamins in the diet are of great importance in terms of antioxidant defence, it should be born in mind that antioxidant vitamins are only one of many protective antioxidant pathways in addition to other endogenous free radical scavengers (albumin, urate, and bilirubin) and metal-preventive antioxidants (caeruloplasmin and transferrin) [13]. The major substances in plasma contributing to total antioxidant capacity are albumin, urate, ascorbate, alpha tocopherol, and bilirubin [14]. Therefore measurement of plasma total antioxidant capacity may give a more precise indication of the relationship between antioxidants and disease Vinychuk et al. [15] showed that disturbances in prooxidant-antioxidant balance were more prominent in patients with MS and fatigue and included the prevalence of LPO products (free radicals) content over the capacity of antioxidant systems to eliminate them. Such disturbances were less prominent in patients with MS without fatigue. Progression of the demyelination process and increase of the MS severity enhanced the intensity of LPO in patients with fatigue, manifested by increased levels of primary LPO products. Patients with secondary-progressive MS and fatigue had the most prominent disturbances of prooxidant-antioxidant balance. Karg et al. [16] reported that the plasma lipid peroxides expressed in terms of thiobarbituric acid reactive substances (TBARS) levels were increased followed by decreased tocopherol level in MS patients. Studies that have evaluated antioxidant in cerebrospinal fluid have reported no differences in alpha tocopherol and serum coenzyme Q10 levels between MS patients and control [17, 18]. In the present study, we demonstrated a significant decrease in plasma total antioxidant capacity in patients with MS compared to the controls. Our results are in accordance with the results of some previous studies that suggested possible role of free radicals in the pathogenesis of MS [9,10]. Our results are in accordance with the results of Sapcanin A. et al. that showed imbalance between production of free radicals and the neutralization by a complex antioxidant system in postmortem cortex in patients with Alzheimer's disease (19). Observed decrease in TAC in MS patients may support the notion that there is, in fact, a strong relationship between impairment of antioxidant defence in the pathogenesis of MS. Our study raises the interesting question of the value of antioxidant treatment in disease course.

Conclusion

Our results showed that oxidative stress play an important role in pathogenesis of multiple sclerosis and suggest the importance of antioxidants use in diet and therapy in MS patients.

Declaration of interest

There is no conflict of interest in this study.

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