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Lipoprotein Oxidation, Plasma Total Antioxidant Capacity and Homocysteine Level in Patients with Multiple Sclerosis

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Free radical-mediated peroxidation of biological molecules, especially of lipids, is implicated in the pathogenesis of a number of diseases like multiple sclerosis. Low concentration of antioxidant vitamins: beta carotene, retinol, alpha tocopherol and ascorbic acid have been observed in serum or cerebrospinal fluid of multiple sclerosis patients. On the basis of these observations, we studied the potential lipoprotein oxidation and total antioxidant capacity in the pathogenesis of multiple sclerosis. Lipoprotein oxidizability for plasma *in vitro*, serum levels of autoantibodies against oxidized low-density lipoproteins, plasma total homocysteine levels with vitamin B_{12} and folate, and plasma total antioxidant capacity were measured in twenty four patients with multiple sclerosis and twenty four healthy sex- and age-matched person as control. In multiple sclerosis patients during an attack, a significant increase in both *in vitro* lipid oxidizability for plasma and in the levels of autoantibodies against oxidized low-density lipoproteins, and a strong decrease in plasma total antioxidant capacity were detected. Plasma total homocysteine levels were significantly higher in multiple sclerosis patients whose plasma vitamin B_{12} and folate levels were lower but not statistically significant, than controls. The present study indicates that lipoprotein oxidation may be important factor in the course of multiple sclerosis and *in vitro* measurements of plasma oxidation kinetics as an indication for lipoprotein oxidation might be useful as an additional tool for the clinical diagnosis of multiple sclerosis.

Keywords: Antioxidant defense; Folate; Homocysteine; Lipid peroxidation; Multiple sclerosis; Total antioxidant capacity

INTRODUCTION

Oxidative stress has commonly been implicated in a variety of pathological events, such as cardiovascular diseases, rheumatoid arthiritis, inflammatory disorders, cancer, and neurologic diseases including multiple sclerosis (MS), which is a degenerative auto-immune disorder of the central nervous system. Free radical-mediated peroxidation of biological molecules, especially of lipids, is implicated in the pathogenesis of MS.

The central nervous system is especially vulnerable to oxidative stress because of brain's high oxygen consumption, abundant lipid content, and relative paucity of antioxidant compounds compared with other tissues (Spector, 1977; Bush, 2000). 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. Under normal circumstances, the potential damaging effects of free radical-mediated peroxidation of biological molecules, lipids in particular, are limited by a number of antioxidants (Halliwell and Gutteridge, 2001). In a previous study, we have examined the serum levels of antioxidant vitamins, alpha tocopherol, beta-carotene, retinol and ascorbic acid and of TBARS in MS patients and compared them to levels obtained from healthy controls

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(Besler *et al.*, 2002). The findings of the study as well as others (Calabrase *et al.*, 2000; Noseworthy *et al.*, 2000) may support the idea that there is in fact a strong relationship between antioxidant vitamins and free radical-induced lipid peroxidation in the pathogenesis of MS.

Plasma thiobarbituric acid reactive substances (TBARS) test has been used clinically to investigate radical-mediated lipid peroxidation and oxidative stress in disease states. In spite of its simplicity and sensitivity, however, the TBARS test has been criticized for inadequate specificity, especially when using human plasma. Since lipoproteins in the density range observed in plasma contain polyunsaturated fatty acids (PUFA), the major substrate for lipid peroxidation, as well as lipophilic antioxidants such as alpha tocopherols, changes in the chemical composition of lipoproteins and subsequent lipoprotein oxidation are accepted to indicate the level of oxidative damage (Steinberg *et al.*, 1989; Steinberg, 1997; Halliwell and Gutteridge, 2001). Several attempts have been also made to assess the total antioxidant capacity (TAOC) of body fluids rather than specifically identifying what has happened to each component of the complex antioxidant defense system. Antioxidants within cells, cell membranes, and extracellular fluids can be upregulated and mobilized to neutralize excessive and inappropriate free radical formation (Halliwell and Gutteridge, 2001). Within the strategy to maintain redox balance against oxidant conditions, blood has a central role because it transports and redistributes antioxidants to every part of the body (Ghiselli *et al.*, 2000; Halliwell and Gutteridge, 2001). For example, plasma can scavenge long-lived reactive oxygen species, such as the superoxide anion or hydrogen peroxide, thus preventing reactions with catalytic metal ions to produce more harmful species. It can also reduce oxidized ascorbic acid back to ascorbate (Ghiselli *et al.*, 2000; Halliwell and Gutteridge, 2001). Hence, plasma antioxidants status is the result of the interaction of many different compounds and systemic metabolic interactions. Plasma total antioxidant capacity 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 (known and unknown, measurable and not measurable) than the concentration of single, selected antioxidants.

To the best of our knowledge, no study has investigated plasma TAOC in patients with MS. We therefore performed this study: (1) to determine some biomarkers of lipid peroxidation in plasma or serum as lipoprotein oxidation and lipoprotein oxidation products, (2) to determine plasma levels of the thiol homocysteine, which is considered to be involved in the oxidation of low-density lipoproteins and (3) to investigate plasma total antioxidant capacity, in a group of secondary progressive MS patients who first experience exacerbation, and compared them to levels obtained from healthy controls.

MATERIAL AND METHODS

Patients and Controls

Twenty-four patients (median age: 34 years; range: 31–42 years; 8 females and 16 males) with secondary progressive MS were included in the present study as previously reported (Besler *et al.*, 2002). The diagnosis of MS was made according to the clinical and laboratory diagnostic criteria of the Poser Committee (1983). Twenty four healthy sex- and age-matched persons (median age: 35 years; range: 32–41 years; 8 females and 16 males) who had normal physical examination findings, served as the control group. Secondary progressive MS patients who first experience exacerbations were recruited from outpatients making the first visit to the departments of neurology or the physical therapy and rheumatology of two hospitals based in the region of Ankara. Neurologic status and progression of disease were evaluated by the Kurtzke disability status scoring. The patients and healthy controls had received neither steroid therapy nor vitamin supplementation, and none of them were smokers. Informed consent according to the declaration of Helsinki was obtained from each subject at the time of recruitment to the study. The study was approved by the Research Ethical Committee of the hospitals.

Sample Collection

Ten microliters of ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood was drawn from the antecubital vein of each subject and immediately placed onto ice. At the same time, a 5 ml of blood was also taken for oxidized LDL measurement in serum. Blood was collected after an 12 h of fasting during the morning hours, and centrifuged at 4°C for 10 min at 2500 rpm to obtain either plasma or serum. Plasma and serum samples were freshly frozen under nitrogen, and stored at −70°C until analysis. Samples were not stored longer than 6 months. The samples were thawed at room temperature only once at the time of assay.

Plasma Lipid Profiles

Plasma levels of total cholesterol (TC), triglycerides (TG) and HDL-cholesterol (HDL-C) were measured enzymatically (Allain *et al.*, 1974; Rifai *et al.*, 1998). HDL-C was determined after precipitation of apo B containing lipoproteins with phosphotungstic

acid/$MgCl_2$. LDL-cholesterol (LDL-C) was calculated using the Friedewald algorithm (Friedewald *et al.*, 1972). Plasma levels of TC, TG, HDL-C and LDL-C were expressed as mmol/l.

Plasma Oxidation Kinetics

Plasma oxidation was monitored as a change in the sample absorbance at 234 nm by the method of Esterbauer *et al.* (1989). This parameter has been shown to reflect the level of lipid hydroperoxides in isolated low-density lipoprotein (LDL) oxidized under *in vitro* conditions. Lipid hydroperoxides are other products of LDL oxidation that have conjugated diene structure and specifically absorb at 234 nm. However, they comprise only a small percentage of hydroperoxides formed during lipoprotein oxidation (Lenz *et al.*, 1990). When lipoproteins are oxidized in diluted plasma changes in the absorbance at 234 nm correlate with other indices of lipid peroxidation, such as consumption of polyunsaturated fatty acids (PUFAs) and accumulation of cholesterol fatty acid (linoleate in particular) hydroperoxide (Esterbauer *et al.*, 1989; Regnström *et al.*, 1993). It has been also shown that when oxidized plasma was treated with sodium borohydride, to eliminate hydroperoxides, and then extracted with hexane, no increase in the absorbance of extract at 234 nm was detected in comparison with unoxidized samples (Schippling *et al.*, 2000). These data justify the use of absorbance at 234 nm as a specific measure for the accumulation of lipid hydroperoxides in the lipoproteins. In order to run the assay of plasma oxidation kinetics, plasma was diluted 150-fold with phosphate-buffered saline and incubated at 37°C for 20 h in the absence of exogenous oxidants (auto-oxidation condition) or in the presence of the exogenous oxidant 2,2′-azobis-(2-amidinopropane) hydrochloride (AAPH) at 330 μM (Kontush and Beisegel, 1999). The absorbance at 234 nm was continuously monitored at 5-min intervals over 20 h at 37°C in sealed quartz cuvettes to prevent evaporation, by using a UV/VIS spectrometer (ATI Unicam-8700, Cambridge, Great Britain) and the formation of conjugated dienes was quantified by the mean oxidation rate during the initial linear phase. Plasma oxidation rate was expressed as nM dienes/l/min.

Autoantibodies Against Oxidized Low-density Lipoproteins in Serum

As expected from the complexity of lipid peroxidation, different assays give different results and it is therefore better to use at least two different assay methods when studying lipid peroxidation. Recent evidence suggests that autoantibodies against oxidatively modified LDL can be used as a parameter that consistently mirrors the occurrence of oxidation processes taking place *in vivo*. In the present study, this index of lipid peroxidation was measured by oLAB kit (Biomedica, Austria). The oLAB kit is an enzyme immunoassay designed to determine human autoantibodies to oxidized LDL directly in serum. In this method, Cu^{++} oxidized LDL is coated onto microtiter strips as antigen. Autoantibodies, if present in prediluted serum, bind specifically to the antigen. After a washing step, a specific peroxidase conjugated anti human IgG antibody detects the presence of bound antibodies. After removal of unbound conjugate through washing, tetramethylbenzedine is added to the wells as a non-toxicchromogenic substrate. The concentration of specific IgG in the sample is quantitated by an enzyme-catalyzed colochange detectable on the ELISA reader (Molecular Device, Thermomax, USA) at 450 nm against 650 nm as reference. A calibration curve was constructed from the standards. Results of the samples were read from this calibration curve, and expressed as mU/ml. The intra- and inter-assay variations were 3.1 and 4.6%, respectively.

Plasma Total Homocysteine Levels and Related Measurement

Plasma total homocysteine (tHcy), which includes the sum of protein-bound and free homocysteine, was assayed based on high-performance liquid chromatography (HPLC) of the fluorescent 7-benzo-2oxa-1,3-diazole-4-sulfonic acid (SBD-F) derivative (Dudman *et al.*, 1996). The derivatization of plasma Hcy was performed according to the method of Ubbink *et al.* (1991). Briefly, the plasma samples (200 μl) were supplemented with an internal recovery standard *N*-acetylcysteine (1.1 mM, 20 μl), treated with tri-*n*-butylphosphine solution (100 g/l in dimethylformamide, 20 μl), and incubated at 4°C for 60 min before precipitation with 10% trichloroacetic acid (in 1 mM of EDTA, 200 μl). One hundred microliters of supernatant was obtained by centrifugation and incubated with borate/EDTA buffer (0.125 M, pH 9.2, containing 4 mM of EDTA, 250 μl), NaOH solution (1.5 mM, 20 μl), and SBD-F solution (100 μl of a 1-g/l solution in borate/EDTA buffer) for 60 min at 60°C and then cooled to room temperature. All derivatized samples were filtered before HPLC. Samples (20 μl) were injected onto a reverse-phase HPLC column (C18-5μ; 250 × 4.6 mm, Phenomenex, USA). With a Spectrasystem SCM100 (Thermo Separation Products, TSP) pump, isocratic elution occurred at 1.2 ml/min with potassium phosphate buffer (0.1 M) containing 35 ml/l of CH_3CN, adjusted to pH 3.5. The detector was a spectrasystem FL3000 fluorescence detector (TSP). Fluorescence detector excitation and emission wavelengths were 385 and 400 nm, respectively. Data were

collected and analyzed with a PC1000 software (ThermoQuest, USA), and expressed as μmol/l.

Plasma vitamin B_{12} and folate were measured by using a commercially available kit (Simultrac, ICN Flow, Irvine, United Kingdom). Results for plasma vitamin B_{12} and folate were expressed as pmol/l and nmol/l, respectively. Intra-assay CVs for plasma vitamin B_{12} and folate were 2.4 and 3.6%, respectively. Inter-assay CVs for plasma vitamin B_{12} and folate were 4.6 and 5.8%, respectively.

Plasma Total Antioxidant Capacity

Plasma TAOC was measured using the Azino-diethyl-benzthiazoline sulphate (ABTS) method (Miller *et al.*, 1993). In this assay, incubation of ABTS with H_2O_2 and a peroxidase (metmyoglobin) resulted in the production of the blue–green radical cation $ABTS^+$, which was measured at 600 nm. Antioxidants in test plasma resulted in suppression of this color production, which was proportional to their concentration. The system was standardized using Trolox, a water-soluble vitamin E analogue. Results were expressed as mmol Trolox equivelent/l of plasma and intra-assay and inter-assay coefficients of variation at 1.4 mmol/l were 1.3 and 2.8%, respectively.

Statistical Analysis

Overall group comparisons (patients with MS *vs* controls), involving continuous variables that were not normally distributed, were analyzed with use of Mann–Whitney *U* test. Spearman's Rank Correlation Coefficient analysis was used to investigate the relationship between two quantitative variables. All statistical analyses were performed using "SPSS 10.0 for Windows" (SPSS Inc., USA). Categorical variable (gender) was also analyzed with use of the Wilcoxon signed rank test. Since there were no differences in the parameters measured, the data were not strafied with respect to gender. Data are presented as median and mean ± SD. A significance level (p value) of 5% was considered statistically different unless otherwise stated.

RESULTS

The clinically diagnosed MS patients and controls were well matched for age and sex. There was no significant difference in both median and mean ages of the patients and healthy controls. Pertinent laboratory data for patients with MS and the control group are summarized in Table I.

There was no significant difference between the plasma levels of lipid profile in patients with MS and controls (Table I).

The mean plasma oxidation rate observed in the control group was in accordance with previously reported data by Kontush and Beisiegel (1999). In this method, a typical time course of the absorbance at 234 nm exhibited three consecutive phases: the *lag phase*, during which the oxidation rate was very close to zero; the *propagation phase*, representing rapid accumulation of lipid peroxides; and the *plateau phase* with an oxidation rate close to zero again. When the mean oxidation rate during the initial phase, expressed as nanomoles of dienes liter per minute (nM/l/min), in the absence of exogenous oxidant (auto-oxidation) or in the presence of the exogenous oxidant AAPH were compared, the rates in patients with MS were significantly higher compared to the control group ($p < 0.005$). AAPH resulted in an increased plasma oxidation rate in patients with MS (95% CI of the difference: 10.50–13.72 nM dienes/l/min) and control subjects (95% CI of the difference: 5.51–8.21 nM dienes/l/min) compared to their corresponding oxidation rates at the auto-oxidation condition (95% CI of the difference: 4.34–5.56 nM dienes/l/min and 1.08–2.68 nM dienes/l/min, respectively) (Fig. 1a,b).

Serum autoantibodies against oxidized low-density lipoprotein (AB-OxLDL) concentrations were higher in MS patients than they were in the control group ($p = 0.005$). However, serum AB-OxLDL

TABLE I Biochemical variables in patients with multiple sclerosis and in the control group

	MS patients (n=24)			Control (n=24)			
Parameters	Range	Median	Mean ± SD	Range	Median	Mean ± SD	p-Value
Triglycerides (mmol/l)	1.78–1.89	1.81	1.83 ± 0.21	1.77–1.91	1.83	1.86 ± 0.26	NS
Total cholesterol (mmol/l)	4.79–5.12	4.88	4.97 ± 0.13	4.69–5.17	4.91	4.92 ± 0.14	NS
LDL-C (mmol/l)	3.14–3.41	3.24	3.27 ± 0.13	3.12–3.38	3.26	3.19 ± 0.16	NS
HDL-C (mmol/l)	1.12–1.34	1.20	1.22 ± 0.16	1.13–1.39	1.22	1.26 ± 0.12	NS
AB-OxLDL (mU/ml)	1318–63679	9841	14305 ± 13548	134–5034	956	1201 ± 1070	0.005
Total homocysteine (μmol/l)	16.13–18.03	16.50	17.08 ± 2.25	11.50–13.18	12.1	12.34 ± 1.98	0.041
Vitamin B12 (pmol/l)	148–295	219.9	223 ± 32	151–348	244.3	243 ± 42	NS
Folate (nmol/l)	11.6–33.8	18.10	18.8 ± 4.08	9.70–30.80	20.85	20.8 ± 3.81	NS
Total antioxidant capacity (mmol/l)	0.88–1.83	1.38	1.39 ± 0.15	1.38–2.15	1.85	1.85 ± 0.14	0.039

NS: not significant.

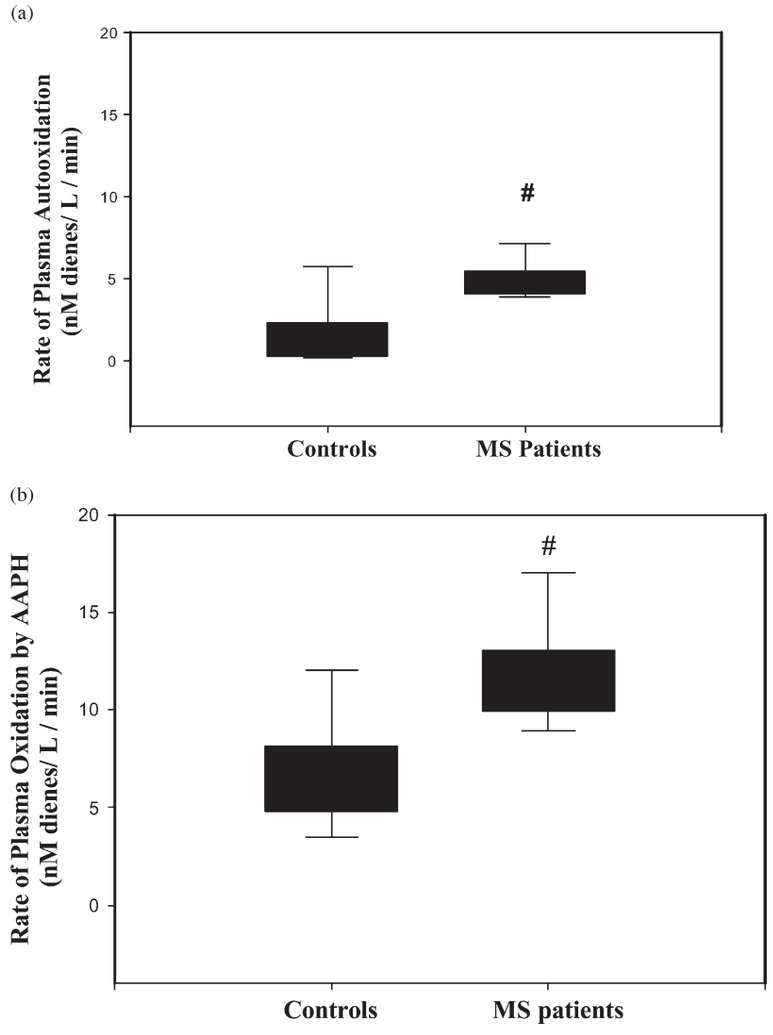

FIGURE 1 Rate of auto-oxidation (a) and AAPH-induced oxidation (b) of plasma obtained from MS patients and control subjects. Plasma was diluted 150-fold with PBS and incubated at 37°C in the absence of exogenous oxidant (autooxidation condition) and in the presence of AAPH (330 μM). $^{\#}P < 0.05$ *vs* control subjects.

concentrations showed high variability in both groups (Table I). Significant positive correlations were observed between AB-OxLDL concentrations and the plasma oxidation rate with or without AAPH ($r_s = 0.46,\ p < 0.05$ and $r_s = 0.53,\ p < 0.05$, respectively).

Plasma total homocystein levels were higher ($p = 0.041$) in patients with MS whose plasma vitamin B_{12} and folate levels were lower, but not statistically significant, than healthy controls (Table I). There was positive correlation between plasma total homocysteine level and serum AB-OxLDL concentrations ($r_s = 0.45,\ p < 0.05$). Correlation analysis revealed that there was no association between plasma total homocysteine level and plasma vitamin B_{12} or plasma folate levels.

MS patients had significantly lower plasma total antioxidant capacity (1.39 ± 0.15 mmol Trolox equivelent/l) compared to the control group (1.85 ± 0.14 mmol Trolox equivelent/l) ($p = 0.039$). Plasma TAOC was inversely, but modestly, correlated with the plasma oxidation rate in the absence ($r_s = -0.59,\ p < 0.05$) or the presence of exogenous oxidant ($r_s = -0.53, p < 0.05$) and serum AB-OxLDL concentrations ($r_s = -0.51,\ p < 0.05$) and plasma total homocysteine levels ($r_s = -0.50, p < 0.05$).

DISCUSSION

In the present study, we have demonstrated a significant increase in both lipid oxidizability for plasma and in the levels of AB-OxLDL and a strong decrease in plasma total antioxidant capacity in patients with MS compared to the controls. Our results also support previous findings of a significant association between MS and elevated plasma levels of thiol homocysteine without any statistically

significant changes in plasma vitamin B_{12} and folate levels. Taken together, these findings suggest that oxidative stress might take place in the pathogenesis of MS.

Plasma lipoprotein oxidation has been extensively studied (Steinberg *et al.*, 1989; Steinberg, 1997) and it has been shown that oxidative modification of plasma lipoproteins plays a crucial role in the pathogenesis of atherosclerosis (Parthasathy *et al.*, 1990; Mohr and Stocker, 1994; Staprans *et al.*, 1994; Steinberg, 1997). The oxidizability of plasma of LDL has been implicated in the development of not only atherosclerosis but also several other diseases, such as Alzheimer's and Parkinson's disease (Kontush and Beisegel, 1999; Arlt *et al.*, 2002). In the present study, *in vitro* oxidizability of plasma was found to be elevated both in the presence (AAPH) and absence of exogenous oxidant (auto-oxidation) in MS patients. In parallel with the mean plasma oxidation rate, we also demonstrated the higher serum levels of AB-OxLDL. We have recently reported that the serum levels of TBARS were increased in MS patients (Besler *et al.*, 2002). Oxidizability of plasma LDL or others can be determined by three major factors: (i) levels of antioxidants; (ii) levels of substrate levels for oxidation, such as polyunsaturated fatty acids, cholesterol or other lipids; (iii) levels of preformed oxidation products or other substances able to accelerate LDL oxidation (Frei and Gaziano, 1993; Kontush *et al.*, 1996; Schippling *et al.*, 2000). Increased plasma oxidizability and serum levels of AB-OxLDL in MS patients might therefore be related to (i) lower levels of antioxidants, (ii) higher levels of substrate for oxidation, and (iii) presence of unidentified oxidants. Although we did not specifically look at the levels of polyunsaturated fatty acids as the main substrate for lipid peroxidation we did not find any changes in the plasma levels of lipid profile in patients with MS and the healthy controls.

Recent studies have shown an increased risk of macrocytosis, low serum and/or CSF vitamin B_{12} levels, elevated plasma tHcy and raised unsaturated R-binder capacity in MS (Sigal *et al.*, 1987; Reynolds *et al.*, 1991; 1992). However Rio *et al.* (1994) reported that no significant differences were found with regard to serum homocysteine levels between MS patients and controls. Nijst *et al.* (1990) measured vitamin B_{12} and folate concentrations in serum and CSF in 293 patients with neurological diseases, including those with MS. They demonstrated that vitamin B_{12} in serum and CSF showed a positive correlation but folate levels did not. This study may imply that serum levels of vitamin B_{12} can be an indicator of CSF vitamin B_{12} in MS patients. Further studies are required to delineate the relationship. Folate and vitamin B_{12} are required both in the methylation of homocsyteine to methionine and in the synthesis of S-adenosylmethionine and are involved in numerous methylation reactions involving proteins, phospholipids, DNA, and neurotransmitter metabolism (Bottiglieri, 1996; Selhub, 1999; Diaz-Arrastia, 2000). Both folate and vitamin B_{12} deficiency may cause similar neurologic and psychiatric disturbances including depression, dementia, and a demyelinating myelopathy (Bottiglieri, 1996). In the present study, the levels of plasma tHcy were significantly higher in MS patients compared to controls. Although the plasma vitamin B_{12} and folate levels were lower in MS patients no significant differences was observed between patients with MS and controls. It is therefore tempting to think that an increase in the plasma tHcy levels in secondary progressive MS patients during an attack might not be attributable to vitamin B_{12} or folate deficiency. Since renal function was normal in MS patients, the cause and importance of an elevated plasma tHcy levels in MS patients, at the present time, remains to be explored. However, it was suggested that the thiolactone form of homocysteine may react with lipoproteins, mainly with LDL, to produce an aggregated and dense form of lipoprotein, which in turn is taken up avidly by macrophages resulting in foam cell formation (Naruszewicz *et al.*, 2001; Chan *et al.*, 2002). Thus, an elevated *in vitro* oxidizability of plasma and serum levels of AB-OxLDL in the present study, and serum TBARS levels as previously reported (Besler *et al.*, 2002) in MS patients, might be one of the consequence of elevated tHcy levels. In addition, homocysteine may have a neurotoxic effect by activating the *N*-methyl-D-aspartate receptor, leading to cell death (Lipton *et al.*, 1997) or it might be converted into homocysteic acid, which also has an excitotoxic effect on neurons in the cerebral cortex (Beal *et al.*, 1991).

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 (Calabrase *et al.*, 2000; Noseworthy *et al.*, 2000). It has been suggested that there is possible role of free radicals in the pathogenesis of MS (Hunter *et al.*, 1985; Bö *et al.*, 1994; Calabrase *et al.*, 2000). Under normal circumstances, the potential damaging effects of these free radicals are limited by the endogenous antioxidant defenses in body (Gey, 1998). Dietary antioxidants such as vitamins appear to be of great importance for the control of the effects of reactive oxygen species. We reported that retinol, beta carotene, alpha tocopherol and ascorbic acid are significantly lower in MS patients compared to controls (Besler *et al.*, 2002). Although vitamins in the diet are of great importance in terms of antioxidant defense, 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) (Jackson *et al.*, 2002). Therefore measurement of plasma total antioxidant capacity may give a more precise indication of the relationship between antioxidants and disease states. The major substances in plasma contributing to total antioxidant capacity are albumin, urate, ascorbate, alpha tocopherol, and bilirubin, ranked in order of percentage contribution (Miller *et al.*, 1993). In the present study in addition to the increase in lipoprotein oxidizability and tHcy levels in plasma, a decrease in TAOC in MS patients may support the notion that there is, in fact, a strong relationship between endogenous antioxidant system and oxidative stress in the pathogenesis of MS.

It can be concluded that MS, an inflammatory demyelinating disease of unknown origin, is neither genetically fully explained, nor are all risk factors influencing its pathogenesis known. The present study indicates that increased lipoprotein oxidation and tHcy levels in plasma may be additional important factors in the progression of the disease. Our study raises the interesting question of whether or not antioxidant treatment could be of any value in the course of the disease. *In vitro* measurements of plasma oxidation kinetics as an indication for lipoprotein oxidation might be useful as an additional tool for the clinical diagnosis of MS. It can also be of particular interest as biochemical markers for the evaluation of antioxidant treatment of MS patients.

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