Serum and Cerebrospinal Fluid Antioxidant Activity and Lipid Peroxidation in Guillain–Barre Syndrome and Multiple Sclerosis Patients

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ABSTRACT

Oxidative stress through the changes in the levels of reactive oxygen species and antioxidative parameters can cause various neurological disorders. The aim of the present study was to show antioxidant activity (AOA) and malondialdehyde (MDA) levels in affected people with Guillain–Barre syndrome (GBS) and multiple sclerosis (MS). A total of 15 GBS patients, 13 MS patients, and 15 age and sex matched controls were included in this study. MDA and AOA values were determined in both cerebrospinal fluid (CSF) and serum, spectrophotometrically. We have shown an increase in the values of MDA in the CSF of both GBS and MS patients (0.32 ± 0.073 and 0.22 ± 0.06 µmol/L) compared to the control (undetectable levels). Furthermore, a significant decrease in the serum MDA levels was shown in both GBS and MS patients (0.81 ± 0.18 and 0.73 ± 0.18 µmol/L) when compared to the control (1.7 ± 0.46 µmol/L). A decrease was shown for serum AOA in both GBS (1.7 ± 0.21 mmol/L) and MS patients (2.6 ± 0.62 mmol/L) when compared to the control (3.2 ± 0.17 mmol/L). However, a significant increase in the values of CSF AOA was shown in both MS and GBS patients (1.47 ± 0.19 and 1.42 ± 0.26 mmol/L) compared to the control (0.71 ± 0.19 mmol/L). An imbalance between the levels of AOA and MDA in both CSF and serum can be followed in both MS and GBS patients.

KEYWORDS: antioxidant activity, cerebrospinal fluid, Guillain–Barre syndrome, malondialdehyde, multiple sclerosis, serum

INTRODUCTION

Organisms have developed several defense mechanisms against oxidative damage. These defense mechanisms are composed of enzymatic and nonenzymatic systems (Arita, Sato, Arai, & Inoue, 1998).

Defense systems not only reduce the levels of lipid peroxidation by means of the administration of active endogenous and exogenous compounds, but also prevent lipid peroxidation by means of the conversion of these radicals into less harmful and harmless compounds. (Bourne, Warthes, McGowan, & Laven, 2007; Carletti et al., 2007; Carter et al., 2005) When free radical damage overwhelms compensation of cells, peroxidation occurs and a series of adverse reactions including alteration of cell permeability, impairment of intracellular and extracellular transport systems, and intracellular energy metabolisms are observed (Arita et al., 1998; Carletti et al., 2007). Furthermore, constituting a problem for cells itself, peroxidation is also capable of causing adverse effect on living organisms through intermediate and final products of peroxidation (Carletti et al., 2007; Carter et al., 2005; Choi, Benzie, Collins, Hannigan, & Strain, 2004).

Oxidative stress induces its harmful effect through the formation and effect of oxidatively damaged macromolecules or their degradation products. The level of malondialdehyde (MDA), a final product of lipid peroxidation, is a parameter that is referred to in the assessment of the occurrence and intensity of lipid peroxidation (Dib, Garrel, Favier, Robin, & Desnuelle, 2002; Doba, Burton, & Ingold, 1985). There are strong evidences that the underlying cause of multiple sclerosis (MS) and some other
neurodegenerative disorders is oxidative stress. Moreover, it was shown that oxidative stress is very high during active progressive multiple sclerosis when compared to those individuals whose multiple sclerosis was in remission or when compared with normal controls (Peresedova et al., 2009).

Evidence of elevated oxidative stress does not prove that it is involved in the neurodegeneration that is associated with a disorder. The cell has evolved several defense and repair mechanisms to deal with oxidative stress and associated oxidative damage, but in these conditions, the activities of various antioxidant defense molecules that would normally counteract the injurious effects of ROS are reduced (Kaur & Ling, 2008).

Free radical toxicity due to poorly maintained cellular redox levels are important events that have been related to the pathogenesis of some diseases affecting the central and peripheral nervous system such as epileptic disorders, demyelinating dementia, and Guillain–Barre Syndrome (GBS) patients. Although unlikely to have any diagnostic value, markers of oxidative stress (such as MDA) might be useful for following disease progression and evaluating treatment (Dib et al., 2002).

So far, some components of the antioxidant system and lipid peroxidative markers have been studied in the cerebrospinal or in the serum of affected subjects with GBS and multiple sclerosis (MS). Thus, the objective of this study is to compare data on the lipid peroxidation and antioxidant activity in the serum and cerebrospinal fluid of the GBS and MS patients.

MATERIALS AND METHODS

Thirteen Iranian MS patients according to the Revised McDonald Criteria (McDonald, 2001) and fifteen patients with GBS according to Asbury and Cornblath Criteria (Asbury & Cornblath, 1990) were studied in Department of Neurology of Iranian Center of Neurological Research, during 2007–2008.

Cerebrospinal fluid (CSF) and blood samples were obtained during standard diagnostic lumbar puncture and peripheral vein puncture, respectively. Cell free supernatants of CSF were stored at −80°C until analysis. Serum was prepared by blood centrifugation, 10 min at 3000 rpm, and stored at −80°C until analysis.

Total protein concentrations were measured on the basis of the biuret method, formation of a violet complex between cupric ions and protein (Silverman & Christenson, 1995). Glucose (GLC) concentrations were measured by the glucose oxidase p-aminophenazone method.

Serum and CSF levels of MDA were estimated by the thiobarbituric acid reaction according to the method of Ledwożyw et al. (1986). Briefly, 1 mL of plasma was mixed with 2 mL of freshly prepared TCA-TBA-HCl reagent (30 g trichloroacetic acid, 0.75 g thiobarbituric acid, and 4.2 mL concentrated HCl were mixed and diluted to 200 mL with distilled water) and 1.5 µL butylhydroxytoluene (0.05%). This mixture was boiled for 30 min in a boiling water bath and then cooled to room temperature. n-Butanol extractable layer centrifuged at 3000 × g for 10 min. Supernatant layer was removed and its absorbance was read at 535 nm. The MDA standard curve was obtained by using malondialdehyde bis (Malondialdehyde bis, S4258497 537, Merck Company, Tehran, Iran).

Serum and CSF antioxidant activity was measured according to the method of Koracevic et al. (Koracevic, Koracevic, Djordjevic, Andrejevic, & Cosic, 2001). In this regard, a standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction, leading to the formation of hydroxyl radicals. These reactive oxygen species degrade benzoate, resulting in the release of TBARS (thiobarbituric acid reactive substances). Antioxidants from the added sample cause suppression of the production of MDA or TBARS. This reaction was measured spectrophotometrically (Shimadzu, UV-120-12) at 532 nm and the inhibition of color development was defined as the antioxidant activity (AOA).

Values of MDA and AOA were compared between patients (MS or GBS) and control groups using unpaired t-student test; α in all cases was 5% (p < 0.05).

RESULTS

In GBS patients serum and CSF levels of glucose (97.92 ± 10.12 mg/dL and 60.83 ± 5.68 mg/dL) did not show any significant difference when compared to the control (78.73 ± 5.47 and 47 ± 7.35 mg/dL). Such findings were shown in the MS subjects for glucose levels in the serum and CSF (97.92 ± 10.12 and 63.83 ± 6.68 mg/dL). While protein values of CSF in the MS subjects (23 ± 3.96 mg/dL) did not show any significant difference compared to the control (31.4 ± 11.76 mg/dL), we have shown a significant increase (99.92 ± 23.95 mg/dL) in the GBS patients.

The mean serum AOA levels in the GBS (1.7 ± 0.21 mmol/L) and MS (2.6 ± 0.62 mmol/L) subjects showed lower values when compared to the control (3.2 ± 0.17 mmol/L). Furthermore, CSF AOA levels in the GBS (1.42 ± 0.26 mmol/L) and MS (1.47
± 0.28 mmol/L) subjects showed higher values when compared to the control (0.71 ± 0.19 mmol/L).

In both GBS and MS subjects serum MDA levels (0.81 ± 0.18 and 0.73 ± 0.18 µmol/L) showed lower levels when compared to the control (1.7 ± 0.46 µmol/L). Moreover, while CSF MDA levels were undetectable in the control group, these values showed significant increase in both GBS and MS subjects (0.32 ± 0.073 and 0.22 ± 0.06 µmol/L, respectively).

DISCUSSION

At present study, concomitant increase in the values of CSF MDA and AOA were seen in both patients. Such finding can be because of some changes in the central nervous system. In this respect, Nossinovitch et al. showed changes in some components of CSF in several pathological conditions (i.e. bacterial and viral meningitis, tumors, hemorrhage. etc. (Nussinovitch, Volovitz, Finkelstein, Amir, & Harel, 2001).

The underlying mechanism of these changes can be because of a disturbance in the blood–brain barrier (BBB) which enables plasma MDA or AOA compounds to reach the CSF or the production of some compounds by the brain tissue, white blood cells, or exogenous bacterial sources. In this respect, compromised BBB was shown in MS patients in the sites of inflammatory lesions so that the focal perivascular leakage of gadolinium on MRI is generally considered as a marker of inflammation (Greco et al., 2004).

Development of disturbance in the BBB can be attributed to high oxygen consumption of the brain tissue coupled with relatively low levels of antioxidant defense mechanisms such as glutathione and a high membrane content of polyunsaturated lipids that are easily oxidized (Kumar et al., 2004).

In a study, Sakai et al. argued that the levels of AOA associated enzymes were not paralleled in MS and GBS patients (Sakai, Inoue, Koh, & Ikeda, 2000). In GBS patients the levels of all these enzymes were not increased (Sakai et al., 2000). However, we showed a decrease in serum AOA levels, yet increase in CSF AOA values in both MS and GBS patients.

Ferreti et al. discussed a role of the oxidative stress and lipid peroxidation in the inflammatory process and in the pathogenesis of MS, a degenerative inflammatory disease of the central nervous system, characterized by autoimmune attack to myelin antigens. Reactive oxygen species (ROS) generated in excess primarily by macrophages, have been implicated as mediators of demyelination and axonal damage (Ferreti et al., 2005).

In previous studies, through direct examination of MS plaque, Frohman et al. has revealed increased free radical activity, with decreased levels of important antioxidants such as glutathione and tocopherol (Frohman, Racke, & Raine, 2006). By considering the role of free radical as a causal factor in MS, we found a decrease in serum MDA levels. A reason for this phenomenon may be due to disease stages. In this respect, Karg et al. showed an increase in the serum MDA level during exacerbations (Karg et al., 2004). Moreover, they showed that blood concentrations of oxidized glutathione were likewise elevated, while the ratio of plasma tocopherol plus triglyceride was deceased. In this regard, they discussed that such changes suggest increased free radical production and consumption of the scavenger molecules during the active phase of the disease. Hence, in blood reduced glutathione level was increased during exacerbation and remission as well (Frohman et al., 2006). It seems that lack of an efficient removal of the lipid peroxidation product by a significantly antioxidant system may be responsible for marked increase in MDA levels in the present studies (Kumar et al., 2004).

On the other hand, while some researchers showed a decrease in the levels of AOA with an increase in the levels of lipid peroxidation in plasma, serum, and CSF of MS patients (Besler & Comoglu, 2003; Calabrese, Raffaele, Cosentino, & Rizza, 1994; Hunter, Nlemadim, & Davidson, 1985; Nazlie, Taskiran, Irkec, Kutay, & Pogun, 2002), others failed to show these alterations (Gutowski, Pinkham, Akamnu, Chirico, & Murphy, 1998). These controversies may be related to different clinical conditions and or disease severity of patients.

The present findings show a decrease in serum AO levels in both GBS and MS patients. In this regard, Kumar et al. showed that the level of plasma antioxidant vitamin C has no change in GBS patients and a tendency for the levels to rise in post-treatment samples. Moreover, they discussed that increase of vitamin E in GBS patients may be a compensatory action of vitamin E for the lack of availability of superoxide dismutase/vitamin C (Kumar et al., 2004).

In conclusion, CSF and serum did not show the same changes for parameters of oxidative stress in both GBS and MS patients. Marked imbalances in the levels of CSF MDA and AOA or serum MDA and AOA can be seen in GBS- or MS-affected persons. Monitoring the antioxidant profiles with clinical and radiographic findings will be certainly useful for assessing the prognosis that should be considered in future studies.

REFERENCES


