Original Article

Expression and enzyme activity of MnSOD and catalase in peripheral blood mononuclear cells isolated from multiple sclerosis patients

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Abstract

Background: It is evident that oxidative stress plays a crucial role in etiology of multiple sclerosis (MS). Dysregulation of antioxidant enzymes have been implicated in demyelination and neuronal loss in MS. The aim of this study was to evaluate mRNA expression and activity of manganese superoxide dismutase (MnSOD), and catalase in peripheral blood mononuclear cells (PBMCs) from patients with relapsing-remitting multiple sclerosis (RRMS) and healthy controls. Materials and Methods: We recruited 20 RRMS patients and 20 age-and sex-matched healthy subjects. PBMCs were isolated, RNA was extracted and real time-PCR was used to evaluate mRNA expression of MnSOD and catalase. Enzyme activity of MnSOD and catalase were measured using colorimetric assays. Results: We found a significant increase in mRNA expression and activity of catalase in PBMCs from patients compared with controls, which was accompanied by reduced activity and expression of MnSOD in MS patients. Conclusion: It appears that impaired antioxidant enzymes in term of high activity of catalase and decreased activity of MnSOD are involved in MS pathogenesis, however further studies are needed to establish this concept.

Keywords: Multiple sclerosis, Oxidative Stress, Catalase, MnSOD

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Introduction

Multiple sclerosis (MS), the most common cause of disability in young adults, is thought to be a chronic inflammatory disease characterized by the presence of scattered focal demyelinated lesions in both white and gray matter in all stages of disease [1, 2]. It is also well-documented that clinical heterogeneity is characteristics of MS and one more mechanism is dominant in various clinical course of disease [3].

Although the etiology of MS has not been clearly elucidated, evidence has been emerging that several pathological processes including demyelination and axonal loss play a crucial role in MS pathogenesis [4, 5]. Numerous studies have demonstrated that oxidative stress, resulting from imbalance between reactive oxygen species (ROS) production and neutralization, is a well-recognized mechanism in the initiation and progression of MS [6]. The anti-oxidant
deficiency and high level of oxidative stress biomarkers have been extensively demonstrated in MS patients and also experimental allergic encephalomyelitis experimental autoimmune encephalitis (EAE), a widely used animal model for MS [7-9].

Oxidative stress activates the oxidative stress-defense system to protect cells against deleterious impacts of ROS against tissue damage and to preserve redox balance. The nuclear factor-E2-related factor (Nrf2) antioxidant response element (ARE) pathway is a crucial part of system encoding antioxidant enzymes. Nrf2 is connected to the actin-bound Kelch-like ECH-associated protein 1 (Keap1) in the cytoplasm in physiological conditions, however in situations linked to high production of ROS, Nrf2 separates from Keap1 and translocates to the nucleus, which in turn lead to transcription activation of ARE-regulated genes [10, 11]. Superoxide dismutases (SODs), peroxiredoxins (Prxs), heme oxygenases (HOs), glutathione peroxidases (GPxs), and catalase are well-known Nrf2–ARE-driven genes involved antioxidant defense system [11]. It is of particular importance that up regulation superoxide dismutase and catalase via inducers or viral vectors have been linked to improve disease severity in EAE [11, 12].

The superoxide dismutases (SODs) and catalase comprise the first line of oxidative stress defense system against ROS. SODs catalyze the dismutation of the superoxide radical to oxygen and hydrogen peroxide and catalase is accountable for converting the hydrogen peroxide to water and oxygen. Impaired activity of MnSOD and catalase has been associated with uncontrolled production of ROS and other free radicals.

It has been suggested that oxidative stress may take precedence over inflammatory responses in MS patients. In other hand, the infiltration of inflammatory cells including T-lymphocytes and monocyte-derived macrophages into CNS and consequently production of large amounts of ROS is believed to have an important role in neuronal damage, oligodendrocyte loss and myelin phagocytosis in MS [4, 13, 14]. Therefore, it is rational that peripheral blood mononuclear cells (PBMCs) as a mixed population of immunocompetent cells can be considered as a plausible cellular model in order to evaluate enzymes involved in oxidative stress defense system in MS.

Despite numerous studies examining the gene expression and enzyme activity of antioxidant enzymes in MS patients[14,15], To our knowledge to date no study has been investigated MnSOD and catalase mRNA expression and enzyme activity in MS in PBMC as a readily available source. Hence, we aimed at evaluating the MnSOD and catalase mRNA expression and enzyme activity in PBMCs isolated from MS patients compared with healthy subjects.

**Methods**

**Patients and control subjects.** The population study consisted of 20 MS patients in relapsing-remitting course of disease (RRMS) and 20 healthy subjects. The patients and healthy subjects in this case-control study was between the ages of 20-40 years and were matched in term of age and gender. The patients were recruited from Sina MS Center, Sina Hospital, Tehran University of Medical Science, Tehran, Iran and all patients were diagnosed by a neurologist based on the McDonald criteria. All patients had an Expanded Disability Status Scale (EDSS) score ≤ 6 and had never received any immunomodulatory and immunosuppressive drugs (n=12) and eight patients were not treated with any immunomodulatory and immunosuppressive drugs during previous 6 month. The control group were selected among healthy subjects with no family history of MS and other autoimmune diseases. The inclusion criteria for both groups were: 1. not received any types of antioxidant supplements, anti-inflammatory drugs, and vitamins in the previous 6 months; 2. had no history of acute or chronic infection, malignancy, diabetes, and any clinically significant systemic disease.

The study was approved by the Ethics Committee of Tehran University of Medical Sciences, and we obtained informed written consents from both cases and controls, prior to the study. The peripheral blood samples were taken in heparin-treated tubes after an overnight fast and were immediately used for PBMC isolation.

**PBMC isolation, RNA extraction and cDNA synthesis.** PBMCs were separated using density-gradient centrifugation by Ficoll-Hypaque
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(Lympholyte-H; Cedarlane Laboratories, Hornby, ON, Canada), washed twice with PBS, and stored in 70°C to evaluate mRNA levels and enzyme activity assays. Total RNA was extracted using a Total RNA Extraction Miniprep kit (Viogene, Taiwan) based on the manufacturer’s protocol. The purity and concentration of RNA were assessed using a NanoDrop spectrophotometer. The complementary DNA (cDNA) was synthesized from DNase-treated RNA (1µg) using Revert Aid First Strand cDNA Synthesis kit (Thermo Scientific, Fermentas, USA).

The measurement of mRNA expression of MnSOD and catalase. The mRNA expression was evaluated using real-time PCR in a Rotor Gene real-time thermocycler (Qiagen, Hilden, Germany) using specific primers for MnSOD, catalase and β-actin, purchased from Qiagen in the presence of SYBR Green detection kit (Takara Bio, Ostu, Japan). The specificity of PCR products was confirmed by melting curve analysis for each amplified product and gel electrophoresis.

The measurement of MnSOD enzyme activity. Mn-SOD activity was determined in PBMCs using a SOD activity kit ADI-900-157; Enzo Life Sciences; USA) according to the manufacturer's instructions. The cells were washed with PBS and lysed with 1x cell extraction buffer supplemented with phenyl methyl sulphunyl fluoride (PMSF)(Sigma) as described in kit protocol. MnSOD activity was assessed using preincubation of cell lysate with 1mM potassium cyanide (KCN) to inactivate other SOD isoforms. Protein concentration was determined using the method of Bradford. SOD activity data were expressed as units per microgram of protein.

The measurement of catalase enzyme activity. Catalase activity in PMBCs was measured with the catalase assay kit (ab83464;Abcam, Cambridge, MA) according to the manufacturer's protocol. This kit is based on the function of sample catalase in decomposing H₂O₂ to water and H₂O in the presence of an optimal concentration of H₂O₂. The unconverted H₂O₂ reacts with OxiRed probe to produce a product, which can be measured spectrophotometrically at 570nm. The cells were washed twice with PBS and lysed in the assay buffer (contained in kit) using sonication on ice. The resulting supernatant was collected after centrifugation. The catalase activity was reported as U/mg protein (units of Cat per mg of proteins), which is reversely proportional to the signal generated from product. The catalase activity was expressed based on the protein concentration of each treatment, which is determined by the Bradford assay.

Statistical analysis. All data was analyzed using SPSS 19 (SPSS Inc., Chicago, IL, USA). Comparisons between patients and healthy subjects were done by the Student’s independent t test and results were presented as mean±standard error of the mean (SEM). Comparative CT method was used for analysis of the gene expression. Statistical significance was considered at a P<0.05.

Results

MnSOD and catalase mRNA expression in PBMCs from MS patients and healthy subjects. The results concerning mRNA level of MnSOD and catalase in PBMCs from MS patients and healthy subjects were depicted in figure 1. As shown in this figure, the difference between healthy subjects and patients with RRMS for mRNA expression of MnSOD and catalase was statistically significant (P<0.05). In healthy subjects, mRNA levels of MnSOD were higher compared with patients with RRMS (almost 2 fold change), while we found an approximately 2 fold increase in mRNA expression of catalase in PBMCs from RRMS patients in comparison with that in healthy group.

MnSOD and catalase enzyme activity in PBMCs from MS patients and healthy subjects. The enzyme activity of MnSOD and catalase in PBMCs from Patients with RRMS and healthy subjects were demonstrated in figure 2 and 3, respectively. MnSOD enzyme activity in PBMCs of MS patients (11.75±0.7 U/mg of protein) was significantly lower in comparison with control group (20±2.23 U/mg of protein) (Figure 2). In case of catalase enzyme activity, a marked and significant increase was observed in enzyme activity of catalase in patients with RRMS (11.33±0.63 U/mg of protein) in comparison with healthy group (4.16±0.93 U/mg of protein) (Figure 3).
Discussion

The main findings of our study are as follows: 1) A significant increase in mRNA expression and activity of catalase was observed in MS patients in comparison with healthy subjects; 2) A significant reduction in mRNA expression and activity of MnSOD was found in MS patients compared with controls. These results were in accordance with previous studies regarding the assessment of mentioned antioxidant enzymes in serum, lesions and CSF of MS patients. However, some studies found no significant difference with regard to MnSOD, catalase and others obviously observed different pattern in this regard.

Pathologically, MS is characterized by infiltration of inflammatory cells including T-lymphocytes and monocyte-derived macrophages into CNS. One important consequence of leukocyte infiltration into the brain parenchyma is production of large amounts of ROS which result to neuronal damage, oligodendrocyte loss and myelin phagocytosis [16, 17]. Decrease in activity of SOD by either inhibition or reduced synthesis has been linked to uncontrolled production of oxygen free radicals (ROS), and increased permeability of blood brain barrier (BBB) [8, 18]. In other hand, ROS has been implicated in infiltrating immune cells via increase in BBB permeability. Notably, infiltrating leukocytes, themselves, by production of non-inflammatory mediators such as ROS, can contribute to myelin degeneration and axonal damage in a positive feedback loop [19, 20]. Hence, in light of the fact that decrease in activity of SOD can lead to increased concentration of free radical, particularly, super oxide anions, it is plausible that that the increased activity of catalase can be considered as an probable compensatory response for confronting oxidative stress–mediated damage in MS. Since it has been also demonstrated reduced level of Nrf2-regulated enzymes can exacerbate ROS-mediate tissue injury and contribute to myelin degeneration and glial activation [21, 22], our result of high enzyme activity of catalase can further reinforce this concept.

Despite the considerable evidence linking the activity of antioxidant enzymes with presence and
extent of neuro-inflammation and oxidative injury, a large discrepancy observed in pattern of enzymatic and non-enzymatic antioxidants in MS, may be explained by a number of factors: MS presents a highly heterogeneity in term of clinical course of disease and also extent of severity, hence, it is conceivable that differences in patients included, can lead to distinct oxidative stress pattern. In addition, the choice of control groups is likely to offer a bias in results, and this was partly because all individuals endure mild levels of oxidative stress, particularly, when the control group selected from patients with other non-inflamatory neurological disease. This concept strengthens when the control group selected from patients with other non-inflamatory neurological disease. Moreover, the use of different samples (CSF, peripheral blood cell, MS lesions and plasma) and also different techniques for assessing oxidative stress-related marker can be considered in this regard.

In summary, our observations of dysregulated expression and activity of oxidative stress response-related genes, in PBMC of MS patients are considerable in several ways. Firstly, these results provide novel insights into the importance of regulation of oxidative stress in immune cells such as PBMCs. Secondly; it draws more attention to the regulation of oxidative stress pathway in PBMCs as a putative target for therapeutic intervention in oxidative stress–related disease.

Accordingly, the present study can partly uncover obscure issues involved in role of oxidative stress in MS pathogenesis; however, some limitation of this study merits consideration here. From an ethical point of view, it is not possible to obtain a large amount of blood in order to isolate PBMC, therefore, we could not thoroughly investigate other important anti-oxidant enzymes in MS patients. Also, recruitment of other MS subtypes can be helpful to investigate role of oxidative stress in MS pathogenesis as a disease with highly variable picture. Therefore, further studies are required to fully establish the importance PBMCs in regulation of high oxidative stress-mediated tissue injury in MS as the known autoimmune disease.

**Conclusion**

It appears that impaired antioxidant enzymes in term of high activity of catalase and decreased activity of MnSOD are involved in MS pathogenesis, however further studies are needed to establish this concept.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

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