Measurement of Serum Superoxide Dismutase and Its Relevance to Disease Intensity Autistic Children

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ABSTRACT

Background: Autism is a pervasive disorder and its prevalence increased in recent surveys. An estimated 1 out of every 88 children is affected by autism. Autism disorder symptoms appear before the age of three. It is believed that serum levels of superoxide dismutase may play a role in etiology of autism.

Materials and methods: Between October and November 2014, 27 Iranian children from Mashhad city were selected in this study. Given these assumptions, the amount of SOD serum in autistic patients and healthy individuals and correlation between the amount of SOD and autism severity were examined. Blood samples of 30 autistic children and 18 age-matched healthy children were collected between 9 to 11 am. Serum level of SOD in both groups was measured by ELISA method.

Results: The mean SOD level in the treatment group (1.04 ±1.33 ng/ml) was significantly lower than the control group (p = 0.001). However, SOD level was not significantly associated with the autism severity (p = 0.667). Conclusions: Decreased serum levels of superoxide dismutase in the early diagnosis of autistic children can be considered as a diagnostic biomarker.

Keywords: autism, biomarker, superoxide dismutase
The Role of Superoxide Dismutase in Autism

Background

Autism is a pervasive disorder during infancy, occurring mostly during the first three years of life (1,2). The prevalence of autism has increased in recent decades (1-4). An estimated one out of every 88 children is affected by autism. Autism is generally the inability to communicate with others through eye contact. Autistic children may have trouble connecting with other people or developing language skills, as well as a tendency for repetitive patterns of behavior (5,6). Both genetic and environmental factors are known to play major roles in the development of this condition (7). A high degree of concordance in identical twins and siblings suggests a genetic predisposition to the disease (8). From an epidemiological point of view, autism averages a 4:1-3 male to female ratio (7). Several environmental factors may play a role in the development of the disease such as: exposure to teratogens, stress and age of the mother during pregnancy, low birth weight and premature birth, and postnatal environmental factors (e.g. oxidative stress, vitamin D- heavy metal poisoning). Oxidative stress intensifies the effect of genetic and environmental factors (7). Superoxide dismutase is the key enzyme in dismutation superoxide radicals generated by oxidative stress (9). Evidence suggests that oxidative stress is caused by genetic and environmental factors and immunological factors. Oxidative stress is the result of an imbalance between the antioxidants and oxygen species (ROS) (2). Recently, suggested that may be the incidence of autism is the result of an imbalance between antioxidants. The presence of outer oxidant reduce the number of antioxidant enzymes and antioxidants capacity and to continue this trend leading to the activation of inflammatory mediators by oxidants (10). Low levels of antioxidants in children due to the risk of oxidative stress is more common (2). Environmental factors such as air pollution and heavy metal poisoning expose your body to oxidative stress, which may underlie neurological disorders such as autism (2).

Results of the superoxide dismutase activity are controversial. Some investigators have shown an increased serum level of Cu / Zn SOD in autistic children (11). The natural levels of ceruloplasmin, superoxide dismutase and copper in patients with autism were also compared with the control group, respectively. The lack of a significant relationship between the child and autism spectrum disorder has been reported (12). Superoxide dismutase and glutathione peroxidase levels in autistic children were significantly lower compared with the control group (13). These findings suggest that SOD can be used as a biological marker of autism and may help in the diagnosis of disease severity. The purpose of this study was to evaluate serum levels of superoxide dismutase in autistic children and to investigate the superoxide dismutase levels in relation to the severity of the disease.

Materials and Methods

Screening

In this study, 27 children (aged range: 2 to 13 years) diagnosed with autism were recruited from the autistic child care center. Childhood autism rating scale (CARS) was used to confirm the diagnosis of autism and assess its severity. A 29.5 cut-off point was employed to diagnose autism. Scores of 30–36.5 and 37–60 were classified as mild-to-moderate and severe, respectively. In addition, criteria from the Diagnostic and Statistical and Manual of Mental Disorders 4th Edition (DSM-IV) were also used to differentiate autistic children with CARS >30 from other developmental disorders e.g. Rett, Asperger’s, and childhood disintegrative disorder. Data from this sample were collected between October and November 2014 (14). All patients were Iranian and enrolled in the Noor Hedayat Center of Autism Spectrum Disorders. 18 healthy age- and sex- matched children with an IQ of 90 to 110 were also enrolled as a control group. Study design was in accordance with the tenets of the Helsinki Declaration of 1975, as revised in 2000, and was approved by institutional review board and ethics committee of Azad University of Medical Sciences, Mashhad Branch. A questionnaire consisting of diseases and medical records and other patient information was collected. Venous blood samples from both the case and control groups were taken between 9 to 11 am. Immediately after the formation of a blood clot, it was centrifuged for 10 min at 4°C, (g × 1000). The serum of patients were immediately separated from microtubes (Eppendorf tubes) and then frozen at -70°C until used. Serum SOD levels were measured using an ELISA
commercial kit according to the manufacturer’s protocol (BiOsci-ence company, USA). Samples and standards were run in duplicate, and the sensitivity of the assays was 0.04 ng/ml for SOD.

Statistical analysis

To investigate the relationship between serum levels of superoxide dismutase with different intensities of autism, a normal distribution with intensity superoxide dismutase enzyme in the group of patients and the normal distribution (p > 0.05). Analysis of variance (ANOVA) was performed to compare the mean serum levels disease severity. Data (i.e. mean age, gender, family history and clinical signs and symptoms of autistic children) were analyzed using Statistical Package for Social Sciences (SPSS 18, IBM Corporation, New York, USA). Chi-square test and Student’s t-test were performed to analyze qualitative and quantitative variables, respectively. For non-normal distributions Mann–Whitney test were used. Correlation between serum SOD level and autism severity was determined using the Pearson’s rank correlation coefficient. A p-value <0.05 was considered statistically significant.

RESULTS

Mean body mass index (BMI) was 18.23±4.83 Kg/m² and 20.96±3.25 Kg/m² in autistic children and control group, respectively. Also, Gender distribution, BMI and mean age between the two groups were not significantly different (Table 1). The gender was matched between groups; from the total of 27 autistic children, 6 were females (22.3%) and 21 were males (77.7%), and from the control group, 8 were female (44.4%) and 10 were male (55.6%). History and clinical examination of autistic children was evaluated in Table 1. Based on CARS, 10 children (37%) had mild-to-moderate autism, and 17 children (63%) were with severe form (Table 1).

As shown in Figure 1, serum levels of superoxide dismutase in autistic children were significantly decreased compared with the control group (1.02 ± 1.40 ng/ml vs 1.67 ± 0.90 ng/ml, p <0.001). Also, the mean concentration of SOD was 0.57±0.46 ng/ml in mild-to-moderate and in severe autistic children was 0.56±0.29 ng/ml. However there was no significant change in SOD concentration between mild-moderate and severe autism, but as shown in Figure 2, there was a positive correlation between SOD concentration and autism severity (r = 0.316; p = 0.107).

DISCUSSION

In this study, serum levels of superoxide dismutase were significantly decreased in patients with autism. In the present study, serum levels of superoxide dismutase in autistic children showed a significant reduction (1.02 ± 1.40 ng/ml) in comparison with the control group (1.67 ± 0.90 ng/ml). Severity of autism was examined in relation to the amount of superoxide dismutase. According to CARS, case group was divided into two groups of mild-to-moderate and severe. The correlation between each of these with the level of superoxide dismutase was reviewed. Considering the severity of illness among autism spectrum, no significant correlation was observed with the amount of superoxide dismutase. The regression curve showed no linear relationship between disease severity and superoxide dismutase (p<0.107). Nevertheless, we found a mild positive correlation between SOD concentration and scores related to autism severity.

Considering the conditions of oxidative stress, antioxidant defense activate, it may be the lack of serum superoxide dismutase in children with autism and the inability to correct the conditions of oxidative stress and antioxidant defense against oxidative damage leading to symptoms autism in children. Therefore, we
can conclude that the reduction of serum superoxide dismutase, along with other factors, such as genetic and environmental factors involved in the disease, may be used as a diagnostic factor.

In the survey was conducted by Essa and et al., in Oman, SOD activity was reduced in autistic children (1). Another study that was conducted by Rousseau and his colleagues in America, was found a significant correlation (p <0.0001) between the level of superoxide dismutase in case and the control groups. In addition, reduction in superoxide dismutase levels in autistic children was noted. Decreased serum level of superoxide dismutase is due to an increased oxidative stress which affects immunological and genetic and environmental factors (5). In a study by Romeo and et al in Mexico, ambient air pollution as an environmental factor causes an increase in the level of oxidative stress (13).

**CONCLUSION**

Briefly, serum levels of superoxide dismutase reduced in autistic children, and it can create the underlying disease. The sex and problems during labor and birth factors can be considered as risk factors for the disease. More importantly, due to the small sample size, we believe the findings of the present study should be tested with a larger number of patients.

**Conflict of interests:** none declared.

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