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Serum levels of SOD and risk of autism spectrum disorder: A case-control study

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ABSTRACT

Background: Autism is a severe developmental disorder with poorly understood etiology. This study examined the clinical significance of serum superoxide dismutase (SOD) level, a marker of oxidative stress, in children with autism spectrum disorder (ASD) and typically-developing children between the ages of 2 and 6 years.
Methods: Ninety-six children diagnosed with ASD and 96 sex and age matched typically-developing children were assessed for serum levels of SOD at admission. S0D were assayed by colorimetry, and severity of ASD was evaluated with the Childhood Autism Rating Scale (CARS) Score. The influence of serum SOD levels on ASD was performed by conditional logistic regression analysis, which allows adjustment for confounding factors.
Results: The median serum SOD levels were significantly ($P < 0.001$) lower in children with ASD as compared to typically-developing children [146 (IQR: 133–165) U/ml and 180 (168–199) U/ml, respectively]. Levels of SOD increased with decreasing severity of ASD as defined by the CARS score ($r = -0.432$, $P < 0.0001$). After adjusting for all other possible covariates, SOD remained can be seen as an independent indictor of ASD with an adjusted odds ratio (OR) of 0.955 (95% confidence interval [CI], 0.942–0.969; $P < 0.001$). Based on the receiver operating characteristic (ROC) curve, the optimal cutoff value of serum level of SOD as an indicator for auxiliary diagnosis of ASD was projected to be 160U/ml, which yielded a sensitivity of 84.7% and a specificity of 71.4%, with the area under the curve at 0.811 (95%CI, 0.747–0.874).
Conclusions: Our data suggests that the decreased serum SOD levels could be implicated in the pathophysiology and progression of autism in Chinese children and can be used as an independent risk indicator of ASD.

1. Introduction

Autism spectrum disorder (ASD) defines a group of common, complex neurodevelopmental disorders. The Centers for Disease Control and Prevention (CDC) released the estimate of the prevalence of ASD among children aged 8 years was that 1 in 68 children in 2010 (Mandell and Lecavalier, 2014). The need to understand the causes of ASD and the underlying pathophysiology have become more acute since the number of diagnosed cases has risen markedly in recent years (Tu et al., 2013).

While the cause of autism remains elusive, autism is considered a multifactorial disorder that is influenced by genetic, environmental, and immunological factors as well as increased vulnerability to oxidative stress (Chauhan and Chauhan, 2015, 2006). Genetic, environmental and immunological risk factors induce the oxidative damage, promote neuronal damage, and reduce methylation activity during synthesis of myelin basic protein, which is fundamental for development of the central nervous system (Smaga et al., 2015). In fact, oxidative stress has also been implicated in the pathogenesis of other neuropsychiatric diseases, including major depressive disorder (Nunes et al., 2013), anxiety disorders (Guney et al., 2014), and obsessive compulsive disorder (Kandemir et al., 2013). Increasing evidence suggests a role of oxidative stress in the development and clinical manifestation of autism (Chauhan and Chauhan, 2006).

Superoxide dismutases (SODs) are antioxidant proteins that convert superoxide to hydrogen peroxide (Tamari et al., 2013). Excessive free radical production or oxidative stress may be involved in the pathophysiology of schizophrenia as evidenced by increased SOD activities (Wu et al., 2014). In clinical trials, the association between oxidative stress and autism has not been established and even present conflicting results. The SOD activity

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Table 1
Characteristics of the autism and typically-developing children.

Variable	ASD	Typically-developing	p-value
Demographics	N = 98	N = 98	
Age (years, SD)	3.85 (1.22)	3.85 (1.22)	–
Man (%)	78 (79.6)	78 (79.6)	–
BMI (kg/m^2,SD)	16.22 (1.65)	16.93 (1.74)	0.041
CARS (IQR)	47 (35–52)	22 (18–27)	<0.0001
The median length of symptom onset to include (SD)	138 (76)	–	–
The median age of symptom onset (SD)	3.4 (1.5)	–	–
Laboratory findings			
SOD (U/ml, IQR)	146 (133–165)	180 (168–199)	<0.0001
Hs-CRP (mg/l, IQR)	4.15 (3.45–5.57)	3.26 (2.08–4.53)	<0.0001
HCY (umol/l,IQR)	17.2 (14.1–19.6)	12.2 (9.6–13.7)	<0.001

Data reflect as percentage, mean (SD) or median (IQR); BMI, body mass index; CARS, Childhood Autism Rating Scale; Hs-CRP; high sensitivity C-Reactive Protein; HCY, homocysteinemia; SOD, superoxide dismutase, ASD, autism spectrum disorder.

was either decreased in plasma (Sogut et al., 2003) and erythrocytes (Yorbik et al., 2002) or increased in plasma (Laszlo et al., 2013) and in erythrocytes (Vergani et al., 2011). It was also demonstrated that mice lacking the fragile X mental retardation protein showed a reduced SOD expression and these mice were more sensitive to oxidative stress and demonstrated behavioral characteristics of autism (Bechara et al., 2009). Therefore, the purpose of this study was to investigate the potential role of SOD in Chinese children with ASD by measuring serum circulating levels of SOD and comparing them with age and gender-matched typically-developing children.

1.1. Patients and method

From January 2014 to September 2015, a total of one hundred and ninety-six Chinese children (98 confirmed ASD cases and 98 their age and gender matched typically-developing children) between the ages of 2 and 6 years were included in this study. Children with ASD were diagnosed as autistic disorder according to Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (McPartland et al., 2012), and excluded all children with another axis I psychiatric disorder or having another chronic medical comorbid condition. The enrolled children with ASD were drug-naive and newly diagnosed by a team consisting of at least a child psychiatrist or a neuropediatrician and a child psychologist. All subjects with autism exhibited symptoms were within the typical triad of autistic traits: communication impairment, social deficits, and ritualistic interests.

Typically-developing children matched for age and gender from a kindergarten were assigned as the control group. All controls were also clinically examined by the pediatricians to exclude the possibility that the controls could have any sub-clinical autistic features. Exclusion criteria for both groups included a diagnosis of malnutrition, the presence of active infection, or known genetic disease. The present study has been approved by the ethics committee of the Cangzhou Central Hospital and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All parents of the participating children gave their written informed consent prior to inclusion in the study.

At baseline, demographic data (age and sex), age of onset, time from onset to included, body mass index (BMI) were obtained. The severity of autistic symptomatology was measured by the Childhood Autism Rating Scale (CARS) score using the Chinese version (Chlebowski et al., 2010) at admission. Blood pressure and dietary patterns were also recorded. Among all the ASD and controls, no abnormal blood pressure level was found and there was no considerable difference between the ASD and the controls with regard to the diet.

Blood samples of participants were obtained in the next morning of the day of inclusion under fasting state. The serum samples were collected and stored at −80 °C until required. Repeated freeze–thaw cycles were avoided to prevent loss of bioactive substances. Serum level of SOD was measured by colorimetry according to the manufacturer's instruction. The coefficients of variation (CVs) of inter-assay and intra-assay for the samples containing 80, 160 and 240 U/ml of SOD were 5.0–7.5% and 6.1–8.7%, respectively. The lower detection limit was 15U/ml and the line range was 15–250U/ml. In addition, the serum level of high sensitivity C-Reactive Protein (Hs-CRP) and homocysteinemia(HCY) were also tested by standard laboratory method.

Results are expressed as percentages for categorical variables and as mean (standard deviation, SD) or median (interquartile range, IQR) for the continuous variables. Proportions were compared using the x^2 test, and the paired t-test or the Mann–Whitney test was used to compare continuous variables between groups as appropriate. Correlations among continuous variables were assessed by the Spearman rank-correlation coefficient. Associations between CARS and serum levels of SOD were also assessed using conditional logistic regression models in multivariate adjustment for possible confounders; ie, age, gender, age of onset, time from onset to diagnosis, BMI, serum levels of Hs-CRP and HCY. The influence of serum SOD levels on ASD was performed by conditional logistic regression analysis, which allows adjustment for above confounding factors. The results are expressed as adjusted odds ratios (ORs) with the corresponding 95% confidence intervals (CIs). Receiver operating characteristic (ROC) curves were utilized to evaluate the accuracy of serum SOD to diagnose ASD. Area under the curve (AUC) was used to measurement the accuracy of the test. All statistical analysis was performed with SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). Two-tailed significance values were used and significance levels were set at 0.05.

2. Results

In our study, 98 children with ASD and 98 matched for age and gender typically-developing children were included. In those samples, 79.6% was boy and the mean age was 3.85 years (SD: 1.22). All children were Chinese Han. The median length of symptom onset to include was 138 days (SD: 76). 5 out of the 98 children with ASD (5.1%) had a family history of ASD. The median CARS score on admission was 44 points (IQR: 37–49). Baseline characteristics of the ASD and typically-developing children were shown in Table 1.

The median serum level of SOD levels was significantly ($P < 0.001$) lower in children with ASD as compared to typically-developing children [146 (IQR: 133–165) U/ml and 180 (168–199) U/ml, respectively; Fig. 1.]. We found that the serum SOD reflected the disease activity of ASD. Levels of SOD increased with decreasing severity of ASD as defined by the CARS score. There was a negative association between serum SOD levels and CARS scores ($r = -0.432$, $P < 0.0001$; Fig. 2.). This negative relationship still persists even adjusted for above possible confounders using conditional logistic