Int. J. Devl Neuroscience 40 (2015) 92–96

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Thioredoxin: A novel, independent diagnosis marker in children with autism

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ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form
14 November 2014
Accepted 18 November 2014
Available online 26 November 2014

Keywords:
Autism spectrum disorders
Thioredoxin
Oxidative-stress
Chinese

ABSTRACT

Background: Oxidative stress increases serum thioredoxin (TRX), a redox-regulating protein with antioxidant activity recognized as an oxidative-stress marker. The aim of this study was to assess the clinical significance of serum TRX levels in Autism spectrum disorders (ASD).
Methods: Eighty patients diagnosed with ASD and 100 sex and age matched typically developing children were assessed for serum TRX content at admission. TRX were assayed with solid-phase sandwich ELISA, and severity of ASD was evaluated with the Childhood Autism Rating Scale (CARS) Score.
Results: The results indicated that the median serum TRX levels were significantly ($P < 0.0001$) higher in children with ASD as compared to typically developing children [17.9(IQR: 10.7–25.8) ng/ml and 5.5(3.6–9.2) ng/ml, respectively]. Levels of TRX increased with increasing severity of ASD as defined by the CARS score. After adjusting for all other possible covariates, TRX still was an independent diagnosis marker of ASD with an adjusted OR of 1.454 (95% CI, 1.232–1.892; $P < 0.0001$). Based on the receiver operating characteristic (ROC) curve, the optimal cut-off value of serum TRX levels as an indicator for auxiliary diagnosis of autism was projected to be 10.6 ng/ml. Further, we found that an increased diagnosis of ASD was associated with TRX levels ≥10.6 ng/ml (adjusted OR 15.31, 95% CI: 7.36–31.85) after adjusting for possible confounders.
Conclusions: Our study demonstrated that serum TRX levels were associated with ASD, and elevated levels could be considered as a novel, independent diagnosis indicator of ASD.

1. Introduction

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders that are defined by behavioral observations, and are characterized by impairments in communication and social interaction along with restrictive and repetitive behaviors (APA, 1994). ASD includes autistic disorder, Asperger syndrome, pervasive developmental disorder and Rett syndrome. The prevalence of parent-reported ASD among children aged 6–17 was 2.00% in 2011–2012, a significant increase from 2007 (1.16%) (Blumberg et al., 2013). The need to understand the causes of ASD and the underlying pathophysiology has become more acute since the number of diagnosed cases has risen markedly in recent years (Tu et al., 2013).

The underlying etiology of ASD is unknown. Empirical studies have estimated that genetic syndromes only account for 6–15% of ASD cases (Schaefer and Mendelsohn, 2008). Exposures to environmental toxicants have been implicated in ASD (Roberts et al., 2007). Research studies in ASD, have started to investigate gene–environment interactions and epigenetic factors, rather than fixed genetic defects (Zhang et al., 2014). Specifically, recent studies have implicated physiological and metabolic abnormalities in ASD, particularly immune dysregulation or inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures ('four major areas')(Rossignol and Frye, 2012).

Immune dysregulation and inflammation has been implicated in ASD (Pardo et al., 2005). Oxidative stress could be a factor in neurodevelopmental disorders might be caused by free radicals such as reactive oxygen species (ROS) (Chauhan and Chauhan, 2006; Ross, 2000). James et al. (2004) suggested that an increased vulnerability to oxidative stress may contribute to the development and

Abbreviations: ASD, autism spectrum disorders; ROS, reactive oxygen species; TRX, thioredoxin; BMI, body mass index; CARS, Childhood Autism Rating Scale; CV, coefficients of variation; WBC, white blood cell count; Hs-CRP, high sensitivity C-reactive protein; HCY, homocysteine; SD, standard deviation; IQR, interquartile range; ORs, odds ratios; ROC, receiver operating characteristic; AUC, area under the curve.

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http://dx.doi.org/10.1016/j.ijdevneu.2014.11.007
0736-5748/

clinical manifestation of autism. Numerous indicators of oxidative stress have been documented previously in the blood from children with autism, including decreased antioxidant enzyme activities, elevated lipid peroxidation and accumulation of advanced glycation end products (Blumberg et al., 2013).

Thioredoxin (TRX) is a multifunctional and ubiquitous protein having a redox (reduction/oxidation)-active disulfide/dithiol within the conserved active site (Jikimoto et al., 2002). TRX has been reported to possess multiple biological functions and to regulate various cellular functions via thiol redox control (Nakamura et al., 1996). *TRX* gene was found to have a novel *cis*-regulatory element responsible for oxidative stress in its promoter region (Taniguchi et al., 1996), and can be strongly induced by oxidative stress such as various oxidative agents, ultraviolet irradiation, and ischemic reperfusion. TRX expression is induced in lymphocytes and keratinocytes by oxidative stress and is also secreted into the bloodstream (Jikimoto et al., 2002).

Previous studies had suggested that serum TRX level is an indicator of oxidative stress (Sumida et al., 2000), immune dysregulation (Griffiths et al., 2014), and inflammation (Al-Gayyar et al., 2011). These findings indicated that TRX may be relevant to the pathophysiology of autism. To the best of our knowledge, no such studies were available. Therefore, the purpose of this study was to investigate the potential role of TRX in Chinese children with ASD by measuring serum circulating levels of TRX and comparing them with age and gender-matched typically developing children. Associations between TRX and clinical characteristics of ASD were also examined.

2. Method

From December 2012 to January 2014, eighty confirmed ASD and 100 typically developing children were included in this study. Patients were diagnosed as having autistic disorder according to clinical manifestations and Diagnostic and Statistical Manual of Mental Disorders, 4th edition (APA, 1994), and excluded all children with another axis I psychiatric disorder or having another chronic medical comorbid condition. The enrolled ASD patients were newly diagnosed by a team consisting of at least a child psychiatrist or a neuropediatrician and a child psychologist, and drug-naïve when included. No subject had any diagnosed genetic, metabolic, or neurological etiology for autistic disorder.

One hundred typically developing children matched for age and gender from a kindergarten were assigned to the normal control group. All control cases were also clinically examined by the pediatricians to exclude the possibility that the controls could have any sub-clinical autistic features. The present study has been approved by the ethics committee of the Linyi People's 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 diagnosis, height and weight were obtained. A body mass index (BMI) was calculated. Routine biochemical tests and severity of symptoms of the patients with ASD were evaluated in all patients at admission. The severity of autistic symptoms was measured by the Childhood Autism Rating Scale (CARS) (Chlebowski et al., 2010) score using the Chinese version. They were also examined by the pediatrician (Gao S.J.) for medical problems including the measurement of blood pressure and for their dietary patterns. Among all the patients and controls, no abnormal blood pressure level was found and there was no considerable difference between the patients and the controls with regard to the diet.

Blood samples of patients and controls were obtained at 8:00 ± 30 AM in the next morning of the day of inclusion under fasting state. 5 ml of blood were placed into a dry clean tube and left to clot at room temperature, and then separated by centrifugation for 15 min. The serum was removed and stored at −80 °C until required. Repeated freeze–thaw cycles were avoided to prevent loss of bioactive substances. Serum levels of TRX was measured in duplicate using a solid-phase sandwich ELISA that uses two highly specific antibodies to human thioredoxin protein; one antibody is precoated onto the thioredoxin ELISA plate and the other antibody is HRP-conjugated (*Immuno-Biological Laboratories Co., Ltd.*, Gunma, Japan) according to the manufacturer's instruction. The inter-assay and intra-assay coefficients of variation (CVs) for the samples containing 25, 50, and 100 ng/ml of TRX were 6.4–9.1% and 7.3–10.2%, respectively. The lower detection limit was 0.43 ng/ml and the line range was 0.43–100 ng/ml. Other biomarkers, such as white blood cell count (WBC), 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. Correlations among continuous variables were assessed by the Spearman rank-correlation coefficient. Associations between CARS and serum TRX levels were also assessed using linear regression models in multivariate adjustment for possible confounders; i.e., age, gender, age of onset, time from onset to diagnosis, BMI, blood levels of WBC, Hs-CRP and HCY. Proportions were compared using the Chi^2 test, and the paired *t*-test or the Mann–Whitney test was used to compare continuous variables between groups as appropriate. The influence of serum TRX levels on ASD was performed by binary 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 TRX to diagnose ASD. Area under the curve (AUC) was calculated as measurements of the accuracy of the test. All statistical analysis was performed with SPSS for Windows, version 19.0 (SPSS Inc., Chicago, IL, USA) and STATA 9.2 (Stata Corp, College Station, TX), R version 2.8.1. Two-tailed significance values were used and significance levels were set at 0.05.

3. Results

In our study, 80 patients with ASD were included. In the study population, 78.75% were male and mean age was 3.82 years (SD: 1.34). All patients were Chinese Han population. Five out of 80 patients had family history of ASD. However, none of the patients had a prenatal gene diagnosis. The median CARS score on admission was 44 points (IQR: 37–49). In addition, the median length of symptom onset to include was 126 days (IQR, 78–168 days). Baseline characteristics of the ASD and normal cases were shown in Table 1.

Our results indicated that the median serum TRX levels were significantly ($P < 0.0001$) higher in children with ASD as compared to typically developing children [17.9(IQR: 10.7–25.8) ng/ml and 5.5(3.6–9.2) ng/ml, respectively; Fig. 1.]. We found the serum TRX reflected the disease activity of ASD. Levels of TRX increased with

Table 1
Characteristics of the autism and control cases.

Variable	Children with autism ($n = 80$)	Control cases ($n = 100$)	*P*-value
Demographics			
Age (years, SD)	3.82 (1.34)	3.79 (1.25)	0.783
Man (%)	63 (78.75)	79 (79.0)	0.912
BMI (kg/m^2, SD)	15.52 (1.54)	16.83 (1.72)	0.016
CARS (IQR)	44 (37–49)	21 (18–25)	<0.0001
The median length of symptom onset to include (IQR)	126 (78–168)	–	–
The median age of symptom onset (IQR)	3.2 (2.5–4.0)	–	–
Laboratory findings			
TRX (ng/ml, IQR)	17.9 (10.7–25.8)	5.5 (3.6–9.2)	<0.0001
WBC ($\times 10^9$/L, IQR)	8.12 (6.43–8.99)	8.05 (6.37–8.77)	0.241
Hs-CRP (mg/dL, IQR)	0.66 (0.33–0.87)	0.33 (0.18–0.46)	0.008
Hcy (μmol/L, IQR)	17.8 (13.7–21.5)	11.4 (8.9–14.1)	<0.001

Data reflect as percentage, mean (SD) or median (IQR); BMI, body mass index; CARS, Childhood Autism Rating Scale; TRX, Thioredoxin; WBC, white blood count; Hs-CRP; high sensitivity C-reactive protein; HCY, homocysteine.