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Serum Brain-derived neurotrophic factor levels in Chinese children with autism spectrum disorders: A pilot study

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) plays a critical role in the pathogenesis of Autism spectrum disorders (ASD). The purpose of this study was to investigate the potential role of BDNF in Chinese children with ASD. Sixty patients (48 male, 12 female) diagnosed with ASD and 60 healthy sex and age control subjects were assessed for serum BDNF content at admission. BDNF were assayed with enzyme-linked immunosorbent assay methods, and severity of ASD was evaluated with the Childhood Autism Rating Scale (CARS) Score. The results indicated that the median serum BDNF levels were significantly ($P < 0.0001$) higher in children with ASD as compared to normal cases [17.6(IQR: 13.7–21.4) ng/ml and 11.5(9.6–13.8) ng/ml, respectively]. Based on the receiver operating characteristic (ROC) curve, the optimal cut-off value of serum BDNF levels as an indicator for auxiliary diagnosis of autism was projected to be 15.0 ng/ml. Further, we found that an increased risk of ASD was associated with BDNF levels >15.0 ng/ml (adjusted OR 10.4, 95% CI: 4.39–29.32) after adjusting for above possible confounders. Our study demonstrated that serum BDNF levels were associated with ASD, and higher levels could be considered as an independent risk factor of ASD.

1. Introduction

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders characterized by social and language deficits, communication skills, and the presence of restricted interests and repetitive behaviors (Sajdel-Sulkowska et al., 2011). The estimated prevalence of ASD varies widely from 1 in 68 for autism to 1 in 750 for the narrowest diagnostic criteria (Gong et al., 2014; Wingate et al., 2014). 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).

Both genetic predisposition and environmental toxins and toxicants have been implicated in the etiology of autism; the impact of these environmental triggers is associated with increases in oxidative stress, and is further exacerbated when combined with genetic susceptibility (Freitag et al., 2010). However, the underlying mechanism or a specific metabolic target relevant to ASD has not yet been identified.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of survival-promoting molecules, plays a vital role in the growth, development, maintenance, and function of several neuronal systems. Skewed expression of BDNF has been linked to psychiatric and neurologic disorders including, epilepsy, Parkinson's disease, Huntington's disease, schizophrenia, and depression (Karege et al., 2005; Ciammola et al., 2007; Green et al., 2011; Connolly et al., 2006). Interestingly, one study suggested that BDNF is trophic for serotonergic neurons, and abnormalities in serotonin levels are the most common biochemical findings in autism (Tsai, 2005). Animal studies suggest that concentrations of BDNF in the CNS and serum are closely correlated (Karege et al., 2002), offering the possibility that concentrations in peripheral blood may be useful as a possible biologic marker for autism.

There have been great controversies in studying relationship between BDNF and autism. Some studies reported that serum BDNF are significantly reduced in autism than in normal controls (Nelson et al., 2006; Hashimoto et al., 2006; Nishimura et al., 2007). While other studies reported higher serum BDNF levels obtained from autistic children compared with controls (Al-Ayadhi, 2012; Miyazaki et al., 2004). Similarly, two studies that have examined BDNF levels in neonatal specimens from individuals later diagnosed with autism have yielded inconsistent results (Abdallah et al., Q2 2013; Nelson et al., 2001). Unfortunately, no such researches were

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available in the Chinese population. Therefore, the purpose of this study was to investigate the potential role of BDNF in Chinese children with ASD by measuring serum circulating levels of BDNF and comparing them with age and gender-matched normal controls. Associations between BDNF and clinical characteristics of ASD were also examined.

2. Methods

From January 2011 to December 2013, a total of one hundred and twenty Chinese children (60 confirmed ASD cases and 60 their age and gender matched control cases) participated in this study after taking consent from their parents. Cases were diagnosed as autistic disorder according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, 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.

Sixty subjects 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), height and weight were obtained. A body mass index (BMI) was calculated. Routine biochemical tests and severity of patients with ASD were evaluated in all patients at admission. The severity of autistic symptomatology was measured by the childhood autism rating scale (CARS) (Schopler et al., 1980) score using the Chinese version. It consists of 15 categories, each rated on a four-point scale. The individual is considered mild-to-moderately autistic when his total score falls in the range of 30–36, and severely autistic when his total score falls in the range of 37–60. The normal cases have a total score fall in the range of 15–29.

Blood samples of patients and controls were obtained at 7:00 AM in the next morning of the day of inclusion under fasting state. 2 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. To increase accuracy, all samples were analyzed twice in two independent experiments to assess inter assay variations and to ensure reproducibility of the results ($P > 0.05$). Serum BDNF serum levels were measured with sandwich-ELISA, using a commercial kit according to the manufacturer instructions (DuoSet ELISA Development, R&D Systems, Inc., USA). The lower detection limit was 1.6 ng/ml and the line range was 1.6–50 ng/ml. The intra-assay coefficient of variation [CV] and inter-assay CV were 3.8–6.9% and 4.6–7.7%, respectively.

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. Proportions were compared using the χ^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 BDNF levels on ASD was performed by binary logistic regression analysis, which allows adjustment for confounding factors (age, sex and body mass index). 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 BDNF to predict 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). Statistical significance was defined as $P < 0.05$.

3. Results

In the study population, 80.0% were male and mean age was 3.78 years (SD: 1.22). All cases were Chinese Han population. Baseline characteristics of the ASD and normal cases were shown in Table 1. Four out of 60 patients had family history of ASD. But, none of the patients had a prenatal gene diagnosis. The median CARS score on admission was 42 points (IQR: 38–47). While, the median length of symptom onset to include was 130 days (IQR, 90–180 days).

The results indicated that the median serum BDNF levels were significantly ($P < 0.0001$) higher in children with ASD as compared to normal cases [17.6(IQR: 13.7–21.4) ng/ml and 11.5(9.6–13.8) ng/ml, respectively; Fig. 1]. Levels of BDNF increased with increasing severity of ASD as defined by the CARS score. There was a significant positive association between serum BDNF levels and

Table 1
Characteristics of the autism and control cases.

Variable	ASD patients ($n = 60$)	Control cases ($n = 60$)	*P*-value
Demographics			
Age (years, SD)	3.78 (1.22)	3.78 (1.22)	–
Man (%)	48 (80.0)	48 (80.0)	–
BMI (kg/m^2, SD)	15.2 (1.5)	16.8 (1.7)	0.012
CARS (IQR)	42 (38–47)	22 (19–26)	<0.0001
The median length of symptom onset to include (IQR)	130 (90–180)	–	–
Laboratory findings			
BDNF (ng/ml, IQR)	17.6 (13.7–21.4)	11.5 (9.6–13.8)	<0.0001

Data reflect as percentage, mean (SD) or median (IQR); BMI, body mass index; CARS, childhood autism rating scale; BDNF, brain-derived neurotrophic factor.

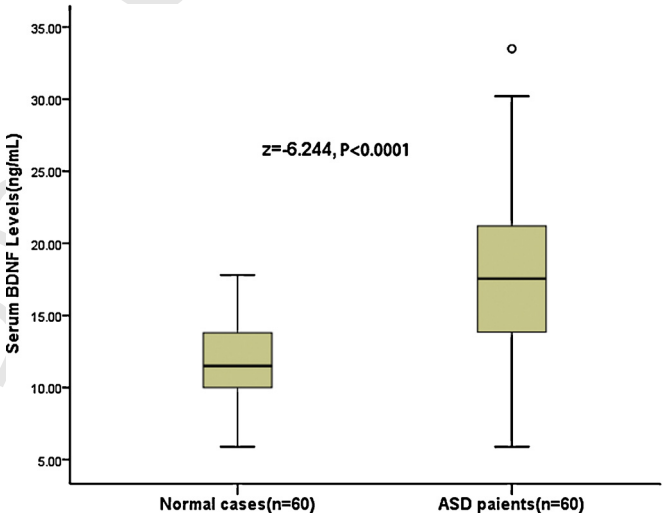

Fig. 1. Boxplot of serum BDNF levels between ASD and normal cases. The serum BDNF levels were significantly ($P < 0.0001$) higher in children with ASD as compared to normal cases.

CARS scores ($r = 0.478$, $P = 0.0006$; Fig. 2). There was also a significant positive associations between serum BDNF levels and time from symptom onset to include ($r = 0.382$, $P = 0.003$). There were no correlation between levels of serum 25(OH) D levels and sex, age and BMI ($P > 0.05$, respectively).

In univariate logistic regression analysis, with an unadjusted OR of 1.42 (95% CI, 1.25–1.63; $P < 0.0001$), BDNF had an association with ASD diagnosed. After adjusting for all other possible covariates, BDNF remained can be seen as an independent predictor with an adjusted OR of 1.33 (95% CI, 1.04–1.51; $P = 0.0002$). Based on the receiver operating characteristic (ROC) curve, the optimal cut-off value of serum BDNF levels as an indicator for auxiliary diagnosis of autism was projected to be 15.0 ng/ml which yielded a sensitivity of 71.7% and a specificity of 86.7%, the area under the curve was 0.830(95%CI, 0.755–0.906) (Fig. 3). Further, in our study, we found that an increased risk of ASD was associated with BDNF levels >15.0 ng/ml (adjusted OR 10.4, 95% CI: 4.39–29.32) after adjusting for above possible confounders. Q3

4. Discussion

In our study, we firstly assessed serum BDNF levels in children with ASD and the correction with severity of autism in Chinese population. Our main finding was that the significantly ($P < 0.0001$)

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