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High plasma neopterin levels in Chinese children with autism spectrum disorders

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ABSTRACT

Background: Neopterin, a pteridine mainly synthesized by activated macrophages, is a marker of inflammation, immune system activation and an active participant in Autism spectrum disorders (ASD). The aim of this study was to assess the clinical significance of plasma neopterin levels in ASD.
Methods: Eighty patients diagnosed with ASD and 80 sex and age matched typically developing children were assessed for plasma levels of neopterin at admission. Plasma neopterin levels were measured using a human ELISA kit and severity of ASD were evaluated with the Childhood Autism Rating Scale (CARS) score.
Results: We found that the mean plasma neopterin level was significantly ($P < 0.0001$) higher in children with ASD as compared to controls. Plasma neopterin increased with increasing severity of ASD as defined by the CARS score. Based on the ROC curve, the optimal cutoff value of plasma neopterin level as an indicator for auxiliary diagnosis of ASD was projected to be 8.5 nmol/L, which yielded a sensitivity of 84.2% and a specificity of 80.1%, with the area under the curve at 0.876 (95% CI, 0.825–0.928). Elevated neopterin (≥8.5 nmol/L) was an independent diagnosis indicator of ASD with an adjusted OR of 12.11 (95% CI: 5.48–28.11; $P < 0.0001$).
Conclusions: These results indicated that autistic children had higher plasma levels of neopterin, and elevated plasma neopterin levels may be associated with severity of ASD among Chinese children.

1. Introduction

Autism spectrum disorders (ASD) are a complex group of neurodevelopmental disorders encompassing impairments in communication, social interactions and restricted stereotypical behaviors (APA, 2000). 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). These figures are in rise as a significant increase in the incidence of autism has been noted in recent years (Depino, 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 (Zhang et al., 2015).

Although current research suggests there may be no single genetic cause for ASD, there are several lines of evidence to suggest that the disorder is highly heritable. Even with the recent advancements in identifying candidate genes involved in ASD, all identified genetic risk factors combined account for only 10–20% of the total ASD population (Abrahams and Geschwind, 2008). A number of these genetic risk factors can also be present in individuals without ASD, suggesting that many of these mutations may increase the risk of developing ASD, but additional risk factors are also necessary (Onore et al., 2012). Research studies have started to investigate gene–environment interactions and epigenetic factors, rather than fixed genetic defects (Zhang et al., 2015).

Recent studies have implicated immune dysregulation and inflammation in ASD. Extensive alterations in immune function have now been described in both children and adults with ASD, including ongoing inflammation in brain specimens, elevated pro-inflammatory cytokincontributiomne profiles in the CSF and blood, increased presence of brain-specific auto-antibodies and altered immune cell function (Onore et al., 2012). Most prior investigations of immune cell counts in autism have focused on monocyte-depleted mononuclear cell samples (Denny et al., 1996). Monocytes circulate in the blood and continually differentiate into macrophages upon migration into the surrounding tissues. Macrophages, in turn, phagocytize pathogens and present antigens to lymphocytes (Sweeten et al., 2003). There is now some evidence that autism may be accompanied by abnormalities in the

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inflammatory response system (IRS). Products of the IRS, such as pro-inflammatory cytokines, may induce some of the behavioral symptoms of autism, such as social withdrawal, resistance to novelty and sleep disturbances (Croonenberghs et al., 2002a).

Neopterin, a pteridine mainly synthesized by activated macrophages, is a marker of inflammation, immune system activation and an active participant in ASD (Sweeten et al., 2003). However, there have been great controversies in studying relationship between neopterin and autism. Previous measurements of neopterin have indicated higher urinary levels in autistic subjects than in comparison subjects (Sweeten et al., 2003). However, plasma levels were lower in autistic subjects than in comparison subjects (Eto et al., 1992). Zimmerman et al. (Zimmerman et al., 2005) found that in cerebrospinal fluid from 12 children with autism, neopterin ($P = 0.003$) were decreased compared with other neurologic disorders. It should be noted that the sample size of above studies were small. Therefore, the purpose of this study was to investigate the potential role of neopterin in Chinese children with ASD by measuring plasma circulating levels of neopterin and comparing them with age and gender-matched normal controls.

2. Patients and method

2.1. Patients and study design

From December 2012 to January 2014, 80 confirmed ASD and 80 typically developing children were included in this study. Children were diagnosed as having ASD according to clinical manifestations and Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (APA, 2000), and excluded all children with another axis I psychiatric disorder or having another chronic medical comorbid condition. The enrolled children with ASD 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. In the 80 children with ASD, 75 children were diagnosed as autistic disorder, 3 as Asperger's disorder and 2 as Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS). No subject had any diagnosed genetic, metabolic, or neurological etiology for autistic disorder.

Eighty typically developing children matched for age and gender from a kindergarten were assigned to the controls. All controls 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.

2.2. Clinical variables

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 a pediatrician (Zhao XH) 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 (Harrison et al. (Harrison et al., 2011) proposed that inflammation and immune activation represented responses to modest elevations of blood pressure that were generally considered benign). Each of the children with ASD and control subjects was medication free for at least 5 weeks and was given a physical examination to screen for evidence of immune activation, such as elevated temperature, infection, inflammation, or malignancy, before blood was drawn.

2.3. Laboratory test

Fasting bloods were collected via venipuncture in ethylenediaminetetraacetic acid (EDTA) BD Vacutainer® (New Jersey, USA) tubes at 7:00 am on the morning after the admission. Blood samples were centrifuged at 1000 × g for 12 min and plasma were separated and stored at −80 °C until the time of assay. Biochemical measurements were done using standard laboratory methods. Plasma neopterin levels were measured using a human ELISA kit (Labor Diagnostika Nord GmbH & Co., KG). The results were expressed as nmol/L. The intra-and inter-assay coefficients of variation were 3.7–6.8% and 3.8–7.7%, respectively. The mean value of morning serum neopterin level in our controls was 5.18 nmol/L. The mean in healthy individuals using this modification was in the range of the reported by another study (5.95 nmol/L in healthy Caucasiansis) (Sweeten et al., 2003). For all measurements, levels that were not detectable were considered to have a value equal to the lower limit of detection of the assay.

3. Statistical analysis

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 Pearson rank-correlation coefficient. Associations between CARS and neopterin levels were also assessed using linear regression models in multivariate adjustment for possible confounders; ie, age, gender, age of onset, time from onset to diagnosis, BMI, blood levels of WBC, monocyte, lymphocyte, Hs-CRP and HCY. Proportions were compared using the chi^2 test, and the paired *t*-test or the Mann–Whitney tests were used to compare continuous variables between groups as appropriate. Receiver operating characteristic (ROC) curves were utilized to evaluate the accuracy of plasma neopterin levels to diagnose ASD. Area under the curve (AUC) was calculated as measurements of the accuracy of the test. The influence of plasma neopterin levels on ASD was performed by binary logistic regression analysis, which allows adjustment for confounding factors ie, age, gender, BMI, blood levels of WBC, monocyte, lymphocyte, Hs-CRP and HCY. The results are expressed as adjusted odds ratios (ORs) with the corresponding 95% confidence intervals (CIs). All statistical analysis were 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.

4. Results

In our study, 88 children with ASD were eligible for the study, and 80 were included (2 parents refused, 3 with axis I psychiatric disorder, 2 with asthma and 1 with vitamin D supplements). However, these 80 patients were similar in terms of baseline characteristics [age ($P = 0.605$), gender ($P = 0.866$), and BMI ($P = 0.890$)] compared to the overall cohort. In the study population, 20% were girls and mean age was 3.69 years (SD:1.30). No abnormalities indicative of immune activation were identified during physical examination or in the screening blood work for any of the children with ASD or controls. 78 out of 80 children with ASD were Chinese Han population. Four out of 80 children had family history of ASD.