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Original Article

Endothelial antibody levels in the sera of children with autism spectrum disorders

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Abstract

Background: The neurobiological basis of autism remains poorly understood. We hypothesized that endothelial antibodies may be associated with the pathophysiology of autism and may predict intellectual/social developmental abnormalities.

Methods: Plasma levels of antiendothelial cell antibodies (AECAs) were measured by enzyme-linked immunosorbent assay in autistic children ($n = 55$) and age-matched healthy controls ($n = 25$).

Results: The serum level of AECAs in children with autism {$n = 55$, 306.4 ± 45.6 pg/mL [mean ± standard error of the mean (SEM)]} was higher (two-tailed Student t test: $p = 0.05$) than that of healthy controls [$n = 25$, 209.6 ± 24.6 pg/mL (mean ± SEM)]. Children with severe autism exhibited significantly higher AECAs than healthy controls (diagnoses of autistic children based on the Childhood Autism Rating Scale score, >40) [$n = 20$, 369.6 ± 65.6 pg/mL (mean ± SEM)] ($p = 0.03$). Disease severity and the Childhood Autism Rating Scale score, which represent stereotyped patterns of behavior in children with autism, were positively correlated ($r^2 = 0.27$, $p = 0.05$).

Conclusion: Elevated AECA serum levels may be implicated in the pathogenesis of autism. However, these data should be interpreted with caution until further investigations are performed using larger sample sizes to determine whether the increase in serum AECA levels is a mere consequence of autism or it plays a pathogenic role in the disease.

Keywords: antiendothelial antibodies; autistic spectrum disorder; autoimmunity; biomarker

1. Introduction

Children with autism exhibit marked deficits in interpersonal social behaviors, abnormal language development, restricted interests, stereotypic/ritualistic behaviors that accompany a particular developmental course, and evidence of developmental delay within the first 3 years of life.[1–4] Autoimmunity to the central nervous system may play a pathogenic role in autism spectrum disorder (ASD).[5–7]

Recently, two lines of research have focused on biological factors in autism. First, excesses of neurotrophins and neuropeptides in infant sera have a predictive value in determining those children who later exhibit disruption of intellectual and or social development.[6] We have established that one of these factors, brain-derived neurotrophic factor, is elevated in children with autism.[8] Second, autoantibodies to several antigenic targets are present in the sera of some children with neurodevelopmental disorders.[9,10] These facts have led us to search for biological markers that may allow earlier detection of autism. In this study, we determined the serum levels of antiendothelial cell antibodies (AECAs) in a group of well-

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characterized children with autism and a healthy control group.

AECAs are observed in various autoimmune diseases, including systemic lupus erythematosus, scleroderma, mixed connective tissue diseases, hemolytic uremic syndrome, Sjogren's syndrome, rheumatoid arthritis, vasculitis, lupus nephritis, Kawasaki disease, and progressive systemic sclerosis.[11–15]

However, serum levels of endothelial cells in patients with autism have never been reported. Thus, the aim of this study was to explore a possible role for AECAs in patients with ASD.

2. Methods

2.1. Participants

This case–control study was conducted with 55 children with classic-onset autism. The autistic group consisted of 47 males and eight females. The children were recruited from the Autism Research and Treatment Center, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia. Their ages ranged between 3 years and 12 years (mean ± SD = 7.98 ± 2.59 years). The patients fulfilled the criteria for the diagnosis of autism according to the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders*.[1] We excluded patients with associated neurological diseases (such as cerebral palsy and tuberous sclerosis) or associated metabolic disorders (e.g., phenylketonuria) because the association of these comorbidities with autism may influence AECA levels. Patients were not receiving any medications.

The control group consisted of 25 age- and sex-matched healthy children (21 males and 4 females). The children's ages ranged between 4 years and 12 years (mean ± SD = 8.79 ± 2.89 years). The children were the healthy older siblings of healthy children who attend the Well Baby Clinic, King Khalid University Hospital, Faculty of Medicine, King Saud University, for routine follow-up of their growth parameters. In addition, informed written consent for participation in the study was given by the parents or legal guardians of the participants.

2.2. Study measurements

Clinical evaluation of autistic patients was based on clinical histories provided by caregivers, clinical examinations, and neuropsychiatric assessments. In addition, the degree of autism severity was assessed using the Childhood Autism Rating Scale (CARS),[16] which rates the child on a scale from one to four in each of 15 areas (relating to people; emotional response; imitation; body use; object use; listening response; fear or nervousness; verbal and nonverbal communication; activity level; level and consistency of intellectual response; adaptation to change; visual response; taste, smell, and touch response; and general impressions). According to the scale, children who score 30–36 have mild to moderate autism ($n = 35$), whereas those with scores ranging between 37 points and 60 points have severe autism ($n = 20$).

2.3. Blood samples

After overnight fasting, blood samples (3 mL) were collected from participants of both groups in plain test tubes. Blood samples were allowed to clot and were then centrifuged at 3000 rpm to collect serum samples, which were stored in a freezer at −80°C until they were analytically assayed. A detailed description of the procedure has been provided in our previous reports.[3,17] To increase accuracy, all samples were analyzed twice in two independent experiments to assess interassay variations and ensure the reproducibility of the observed results ($p > 0.05$).

2.4. Assessment of serum AECAs

Levels of AECAs were measured using a commercially available sandwich enzyme immunoassay (enzyme-linked immunosorbent assay) kit from Cusabio Biotech Co., Ltd (Wuhan, China). No significant cross-reactivity or interference was observed.

2.5. Statistical analysis

The results were analyzed using the commercially available software package Statview (Abacus Concepts, Inc., Berkley, CA, USA). The data are presented as the mean ± standard error of the mean (SEM). The Mann–Whitney *U* test was used for comparisons between data. Spearman's rank correlation coefficient "*r*" was used to determine the relationship between variables. For all tests, $p < 0.05$ was considered significant. The receiver operating characteristic curve is a plot of sensitivity versus 1-specificity at different cutoff values of the studied variable. The uppermost left point represents the best cutoff value (based on the highest sensitivity with the lowest false-positive results of the studied marker) to differentiate between the two groups under study. If the area under the curve is > 0.5, the variable is able to differentiate between the two groups; the closer this area to 1, the better its differentiating capacity. The best cutoff value of serum AECAs was 254 pg/mL, with the area under the curve being 0.64, indicating that plasma AECA was a good differentiating marker between patients and controls at this cutoff value.

3. Results

The general characteristics of the study participants and the results of the AECA levels are depicted in Table 1. Patients exhibited significantly higher serum AECA levels [$n = 55$, 306.4 ± 45.6 pg/mL (mean ± SEM)] ($p = 0.05$, Fig. 1) than did normal controls [$n = 25$, 209.6 ± 24.6 pg/mL (mean ± SEM)]. Increased serum AECA levels were observed in 63% (41/55) of autistic children.

There was a significant difference between normal controls and patients with severe autism (autism diagnoses made on the bases of CARS scores of >40) [$n = 20$, 369.6 ± 65.6 pg/mL (mean ± SEM)] ($p = 0.03$). However, there was no significant difference between the serum AECA levels of children with