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Original Article

Maternal antineuronal antibodies and risk of childhood autism spectrum disorders: A case−control study

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Abstract

Background: The etiology of autism is complex, and may involve the interaction between genetic and environmental factors. Recent studies suggested an association between maternal immune response and this disorder.
Methods: Forty-nine women with autistic children (cases) were studied in comparison with 73 women with normal children (controls). After interviewing for sociodemographic and clinical information, mothers' sera were tested for the presence of antineuronal antibodies.
Results: Mothers of autistic children had significantly higher seropositivity for anti-Yo antibodies (34.7%) than control women (13.7%), with an (adjusted odds ratio of 2.60 (95% confidence interval, 1.03−6.61; $p = 0.044$). Similarly, women with autistic children showed significantly higher seropositivity for antiamphiphysin than the control group (40.8% vs. 17.8%), with an adjusted odds ratio of 2.54 (95% confidence interval, 1.07−6.04; $p = 0.035$). No significant association was found between autism spectrum disorders and maternal anti-Hu antibodies and anti-Ri antibodies, and the history of autoimmune diseases.
Conclusion: Some maternal antineuronal antibodies may contribute significantly to the risk of childhood autism.

Keywords: antineuronal antibodies; autism; autoimmune diseases

1. Introduction

Autism spectrum disorders (ASDs) manifest as highly variable combined deficits in social interaction, and verbal and nonverbal communication, and often include the presence of repetitive, stereotypical, and overly restrictive behaviors.[1] The prevalence has increased substantially over the last decade to approximately 1 in 100 children.[2] The causes of ASDs are unknown, but there is an emerging consensus that ASDs have multiple etiologies, although genetic, neurologic, environmental, and immune factors are likely involved.[3] The symptoms of ASDs are usually diagnosed in early childhood, supporting the current view of a prenatal or early postnatal etiology.[4] Autism develops before the 36th month of age and persists into adulthood, causing lifelong disability.[5]

One proposed cause of ASDs is exposure of the fetal brain to maternal autoantibodies during pregnancy.[6] An etiologic role of maternal antibodies in ASDs is plausible due to the dynamics of the gestational transfer of maternal immunoglobulin G (IgG) during pregnancy. In humans, maternal antibodies are detected in fetal circulation as early as 13 weeks of gestation, and their concentration increases to approximately 50% of maternal levels by 30 weeks of gestation.[7] Many neonatal cases of diseases associated with antibody-mediated autoimmune diseases in the mother are

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documented,[8] e.g., infants born to mothers with thyroiditis suffer from hypothyroidism as a result of maternal antithyroid peroxidase. A mother with systemic lupus erythematous transfers N-methyl-D-aspartic acid receptor autoantibodies to the fetus during pregnancy, causing death of fetal neurons and hence resulting in congenital brain injuries.[9]

Bauman et al[10] reported that the reactivity of maternal antibodies to fetal brain proteins at 37 kDa and 73 kDa is associated with ASDs and certain childhood behavioral disorders. Those researchers then examined whether maternal antibodies related to autism would induce behavioral changes in rhesus monkeys. Three groups of pregnant rhesus monkeys were included in the study: the first group received purified IgG from mothers of children with autism, the second group received purified IgG from mothers of typically developing children, and the third group included untreated controls. Monkeys born to mothers administered with autism maternal serum showed increased whole-body stereotypes across multiple testing paradigms. These monkeys were also hyperactive, while monkeys born to mothers treated with IgG from mothers of normally developing children and untreated monkeys did not show any significant changes in stereotypes.

Various antibrain antibodies have been found in autistic patients, including autoantibodies to serotonin receptors,[11] neuron axon filament protein,[12] myelin basic protein,[13] cerebellar neurofilaments,[14] nerve growth factor,[15] and alpha-2-adrenergic binding sites.[16]

Although paraneoplastic antineuronal antibodies have an association with particular tumors, most commonly small cell lung, breast, and ovarian tumors, they are also detected in patients with neurological syndromes of unknown etiology and occasionally in healthy individuals.[17] Several categories of paraneoplastic antineuronal antibody targets exist. They target either nuclear or cytoplasmic protein antigens such as anti-Yo and anti-Hu, or intracellular synaptic proteins such as antiamphiphysin.[18]

Antineuronal antibodies have been suggested to play a central role in the pathogenesis of neuropsychiatric disorders.[19,20] These disorders can occur in patients with or without cancer—often children or young adults.[18]

Since autism may be one of the pediatric autoimmune neuropsychiatric disorders, this study was conducted to investigate the expression of maternal antineuronal antibodies, as an index of autoimmunity to brain, in autistic children

2. Methods

This case−control study was conducted in the Autism Center, the only private center for autism in Basrah, for the period from August 2014 to November 2014.

Cases were mothers of at least one child with confirmed diagnosis of ASD by pediatric specialists based on the Autism Diagnostic Observation Schedule[21] and the Autism Diagnostic Interview−Revised.[22] Controls were apparently healthy women with normally developing children. Controls were randomly selected from women who were attending a primary health care center. Both groups were without a history of assistant reproductive technique for their childbirth and had no drug intake history. Both groups were frequency matched for age. Data were collected using a questionnaire covering the age of the mothers and children, sex of the child, and the history of autoimmune diseases among mothers during or before pregnancy.

Immunoblotting tests (Ravo Diagnostika, Freiburg, Germany) were used for the detection of anti-Hu, anti-Ri, anti-Yo, and antiamphiphysin autoantibodies in mothers' serum. According to manufacturer's instructions,[23] serum samples were diluted 1:2000 in ready-to-use sample dilution buffer. Strips with 2 mL of the diluted serum specimen were incubated for 60 minutes at room temperature on a rocking table. All strips were washed five times with diluted wash buffer, and then 2 mL alkaline phosphatase IgG conjugate, ready to use, per strip was added. Each strip in 2 mL ready-to-use substrate solution was incubated for 25 minutes at room temperature until the bands became clearly visible. The strips were transferred to distilled water to stop the reaction. Then the strips were placed onto a filter paper for drying and stored the in the dark. Finally, the strips were compared by control scan.

This study was approved by the Ethics and Research Committee of College of Medicine, Basrah University. Written consent was obtained from each mother before being enrolled in the study.

2.1. Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences, version 20 (IBM Corp., Chicago, Illinois, USA). Frequencies and percentages were calculated for categorical variables, and chi-square (χ^2) test was used for assessing the association between these variables. Means and standard deviations (SDs) were measured for quantitative data. Multivariate logistic regression analysis was used to identify the independent risk factors of ASDs. A p value of <0.05 was considered to be statistically significant.

3. Results

The number of mothers with autistic children was 49 (cases), their mean age and SD was 30.0 ± 6.8 years, and the age range of their children was 5−16 years with a mean and SD of 9.9 ± 2.0. The number of mothers with normal children was 73 (controls). Their mean age and SD was 29.6 ± 6.6, and the age range of their children (normal) was 5−16 years, with mean of 10.0 ± 2.1. Boys constituted 63.3% of autistic children, with no significant association between sex and autism (Table 1). Autoimmune diseases (including celiac disease, rheumatoid arthritis, thyroiditis, antiphospholipid syndrome, autoimmune hemolytic anemia, and rheumatic fever) were found in 53.1% of the mothers of autistic children and 60.3% of the mothers of normal children, without significant association ($p = 0.546$).

In univariate analysis, anti-Hu and anti-Ri antineuronal autoantibodies were detected, respectively, in the sera of 32.7% and 32.7% of the mothers of autistic children compared

Table 1
Association of antineuronal autoantibodies and certain risk factors with autism spectrum disorders.

	Cases No. (%)	Control No. (%)	χ^2, p	OR (95% CI)
Gender				0.79 (0.37–1.69)
Boys	31 (63.3)	50 (68.5)	0.359, 0.549	
Girls	18 (36.7)	23 (31.5)		
Anti-Hu Ab				1.59 (0.71–3.58)
Positive	16 (32.7)	17 (23.3)	1.303, 0.254	
Negative	33 (67.3)	56 (76.7)		
Anti-Ri				1.88 (0.82–4.27)
Positive	16 (32.7)	15 (20.5)	2.267, 0.132	
Negative	33 (67.3)	58 (79.5)		
Anti-Yo Ab				3.35 (1.38–8.15)
Positive	17 (34.7)	10 (13.7)	7.500, 0.006	
Negative	32 (65.3)	63 (86.3)		
Antiamphiphysin				3.18 (1.47–7.91)
Positive	20 (40.8)	13 (17.8)	7.866, 0.005	
Negative	29 (59.2)	60 (82.2)		
Maternal autoimmune disease				1.34 (0.67–2.76)
Positive	26 (53.1)	44 (60.3)	0.624, 0.546	
Negative	23 (46.9)	29 (39.7)		

Ab = antibody; CI = confidence interval; OR = odds ratio.

with 23.3% and 20.5% of the mothers without autistic children, with no significant association. However, anti-Yo and antiamphiphysin antibodies were positive, respectively, in the sera of 34.7% and 40.8% of the mothers of autistic children (cases) compared with 13.7% and 17.8% of the controls (mothers without autistic children), with a highly significant association.

The association of anti-Yo and antiamphiphysin with ASDs remained strongly positive after multivariate logistic regression analysis, as shown in Table 2.

4. Discussion

In this analytical study, we assessed the hypothesis that there is an association between maternal neuronal antibodies and childhood ASDs.

This study showed that some of these autoantibodies, anti-Yo and antiamphiphysin, existed significantly more in the sera of mothers with an autistic child than in the sera of mothers of normally developing children. Anti-Hu and anti-Ri were more prevalent in mothers of autistic children than in control mothers, but without a significant association. This result emphasizes the heterogeneity that encompasses autism and the diversity of causative theories that exist.[24] Dalton et al,[6] in their study on mice, proposed a role for maternal antibodies in the etiology of neurodevelopmental disorders, but not necessarily that they are responsible for all cases of autism or other neurodevelopmental disorders.

Table 2
Logistic regression analysis.

Variable	*B*-coefficient	*p*	OR	95% CI	
				Lower	Upper
Anti-Yo	0.957	0.044	2.60	1.03	6.61
Antiamphiphysin	0.933	0.035	2.54	1.07	6.04

CI = confidence interval; OR = odds ratio.

The results of our study are in line with those reported by Passarelli et al,[25] who found a significant positive response to the anti-Yo antibody immunoreactivity in the Purkinje cells of the cerebellum of children with attention deficit hyperactivity disorder (ADHD). Other investigators have identified the presence of antibodies that bind to the brain in mothers with ASD offspring.[26,27]

Zimmerman et al[28] found that specific patterns of antibody reactivity were present in the sera of mothers of autistic children, from 2 years to 18 years after the birth of their affected children, and were unrelated to birth order; they suggested that these autoantibodies could cross the placenta and alter fetal brain development.

Bauman et al[10] and Martin et al[29] examined the morbific potency of these autoantibodies by administrating IgG isolated from the sera of mothers of autistic children to female rhesus monkeys during early and midpregnancy, and then assessed the behavioral and brain development of the offspring. They found alteration in the brain growth, deviation from normal social behavior, and abnormality of growth of the brain. Fox et al[30] reported that maternal antibodies to fetal brain had been identified as one exposure during fetal life that may put a child at risk for ASDs.

Piras et al,[31] in their study on autistic children, found that the presence of patient- and mother-produced antibrain antibodies did not confer an increased risk of autism within the same sibship. However, maternal antibrain antibodies were associated with neurodevelopmental delay in their autistic children.

Maternal antibodies may have a direct antigen–antibody interaction resulting in functional interference of the target proteins, or the presence of these antibodies may be merely a biomarker of cell destruction.[32]

Our study found no significant association between the presence of maternal autoimmune diseases and ASDs, a result also reported by Croen et al[33] who suggested that maternal autoimmune disorders present in women around the time of pregnancy are unlikely to contribute significantly to autism risk. Another study also did not find an association between ulcerative colitis and ASDs.[34] However, a Swedish study[35] revealed that many maternal autoimmune diseases were associated with autism, including Type 1 diabetes mellitus, idiopathic thrombocytopenic purpura, myasthenia gravis, and rheumatic fever.

There are some reasonable and valid ways to explain the effect of the disturbance of maternal immune system on ASDs; it can be through determination of the growth of the brain by initiation of unfavorable surrounding in the uterine cavity,[6,29] or by changing the immunity of the child by the cross of IgG through the placenta, which leads to either temporary or long-lasting structural harm to the child.[36]

One limitation of this study is that we were able to measure autoantibodies in maternal serum only at one point after pregnancy, so it is difficult to prove whether the antibodies that we measured were present earlier or later after pregnancy. This limitation does not rule out the possibility that some of these

maternal autoantibodies had a role in autism, since abnormalities in brain development may not be detected until months to years after birth.[37] In addition, an age-dependent decline in antibody levels is not commonly observed in autoimmune disorders.[38] Furthermore, adult ADHD is defined as ADHD that appeared before the age of 12 years and persists beyond 17 years of age.[39] At that time, the possible causes of brain damage are many. A prospective cohort study of subsequent offspring born to mothers with positive serum paraneoplastic antibodies who already have one child, with or without ASD, will resolve this issue.

In conclusion, the results of this study, along with those of previous studies, support the potential use of mother antineuronal antibodies as biomarkers to predict the risk of ASDs, which may provide new ways to prevent and treat autism.

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