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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 36$^{th}$ 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 ($\chi^2$) 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 $30.0 \pm 6.8$ years, and the age range of their children was 5−16 years with a mean and SD of $9.9 \pm 2.0$. The number of mothers with normal children was 73 (controls). Their mean age and SD was $29.6 \pm 6.6$, and the age range of their children (normal) was 5−16 years, with mean of $10.0 \pm 2.1$. 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