

## SERUM IMMUNOGLOBULINS AND CRP LEVELS IN AUTISTIC CHILDREN

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### ABSTRACT

**Background and Objectives:** The objective of this study was to determine and compare the serum CRP and immunoglobulin levels in autistic children and healthy controls. It was an observational cross sectional study design. Two groups were designed in the study, one for controls and other for patients. Control group include the normal, healthy individuals with no infection, inflammation or immune abnormality. Patient group include the autistic children who fulfil the DSM IV criteria.

**Methodology:** Immunoglobulins (IgG, IgM, IgA) and CRP levels were measured in serum of autistic patients and controls. CRP levels were measured by semi quantitative agglutination method while immunoglobulin levels were measured by immunoturbidity technique. All collected information was entered in to SPSS version 20.0 and analysed through its statistical package.

**Results:** In this study statistically significant elevated serum CRP levels and decreased serum IgG levels were observed in autistic patients as compared to control.

**Conclusion:** The results of this study showed that there are abnormalities in the immune system of autistic patients.

**Key words:** Autism, CRP, Immunoglobulin.

### INTRODUCTION

Autism is a complex neurodevelopmental disorder representing a heterogeneous group of individuals with multiple biologic etiologies. It adversely affects the child's verbal and nonverbal communication, educational performance and social interactions.<sup>1</sup>

About 10 years ago, in America, the prevalence of autism was 4 – 5 children per 10,000 births. But the number of cases has been increased dramatically. Recently, 1 in 125 to 150 children with autism have been reported by the U.S. Centers for Disease Control and Prevention. In other countries, like Canada 200,000 children, in India 1 to 2 million children and in China 1.5 to 3 million children have been reported with autism.<sup>2</sup>

The estimated population of Pakistan is approximately 172,800,048 and out of them approximately 3,45,600 million patients have been reported with autism. Rate of incidence of autism spectrum disorder is 1:120 kids in Pakistan.<sup>3</sup> Estimation of prevalence of autism is variable, depending upon the geographical location, diagnostic criteria and age of children screened. Attention has been focussed on that the prevalence of autism is increasing with time.<sup>4</sup>

This tremendous increase in autism is due to the following reasons: (a) Real increase in the incidence of autism (b) There may be increased case finding, as a

result of increased awareness (c) The diagnosis may be applied more broadly as a result of the changing definition of the disorder, particularly changes in Diagnostics and Statistical Manual of Mental Disorder III (DSM – III) and Diagnostics and Statistical Manual of Mental Disorder IV (DSM – IV).<sup>5</sup>

Although exact etiology of autism is unknown but environmental and genetic factors have important role in the pathogenesis of autism.<sup>6</sup> Immune response changes, presence of autoantibodies against neuronal antigens and involvement of major histocompatibility antigen (MHC) indicates that role of immune system has very significant role in autism. Both adaptive and innate immune system abnormalities such as inflammation, immunodeficiency and autoimmunity have been observed in autistic patients.<sup>7</sup> Another observed cause of autism is childhood vaccines (MMR vaccines) but vaccine hypothesis lack convincing scientific evidence so it is still controversial.<sup>8</sup> In rare cases, autism is strongly associated with agents that cause birth defects.<sup>9</sup>

C-reactive protein (CRP) is an important diagnostic, prognostic or predictive factor for autism. According to the previous investigations, CRP mediates neuroinflammation and directly up-regulates the adhesion molecules on endothelial cells such as VCAM – 1, ICAM – 1 and E-selectin.<sup>10</sup> These adhesion molecules are

responsible for transmigration of leukocytes into inflammatory tissues through endothelium. These findings support the presence of inflammatory cells in autistic brain.<sup>11</sup>

Secondly, CRP is a powerful stimulator of nitric oxide (NO) production by inducible nitric oxide synthases (iNOS). Increased expression of iNOS by CRP leads to the rise in NO concentration which exerts destructive effects; consequently results in the cell death. NO is also chemotactic to neutrophils, eosinophils and monocytes. So, CRP exerts proinflammatory effects, responsible for the pathogenesis of autism. Higher the levels of CRP, greater will be severity of autism which affirms the role of CRP in diagnosis and severity of autism.<sup>10</sup>

Immunoglobulin abnormalities were among the most common immune abnormalities observed in children with autism. It has been reported that decreased total serum levels of IgM and IgG are related to the more aberrant behaviors in autistic patients<sup>12</sup>. The occurrence of a differential antibody repertoire has been studied extensively in autism such as decreased total IgG and IgM levels have been reported. Low levels of immunoglobulins found to correlate with more aberrant behaviors.<sup>13</sup> A low level of IgA also has been observed in autistic patients which results in immunodeficiency.<sup>14</sup> It is suggested that IgA deficiency has been associated with inheritance of C4B null allele (no C4B protein is produced). The C4B null allele is recognized as an extended MHC haplotypes designated (HLA-B44-SC30-DR4). In subsequent study of the MHC it has been found that (HLA-B44-SC30-DR4) was over represented on the chromosomes of autistic subjects as compared to the normal subjects, by about six folds which may be responsible for IgA deficiency.<sup>15</sup>

In summary, it is believed that role of immune system is very important in the pathogenesis of autism. The focus of this study is to find out any relationship between immunity and autism by comparing CRP and immunoglobulin levels of autistic patients with controls.

**Hypothesis:** There are increased serum CRP and decreased immunoglobulins (IgG, IgM, IgA) levels in autistic patients as compared to healthy controls.

**Objective:** To determine and compare the serum levels of immunoglobulins (IgG, IgM, IgA) and CRP in autistic children and healthy controls.

## MATERIALS AND METHODS

This study was conducted in Department of Immunology Children's Hospital and University of Health Sciences Lahore. Total fifty subjects from pediatric age group (2 – 15) were enrolled in this study. Out of these 50 subjects twenty five were autistic Patients and twenty five individuals as control were taken. Control group include the normal, healthy individuals having no inflammatory disease, neurological or psychological

deficits. In case group autistic patients who fulfill the DSM IV criteria were included. Patients who have any other inflammatory, neurological or psychological deficits such as epilepsy, depression, schizophrenia were excluded from patients group. Blood samples of autistic patients were taken from the Rising sun institute Lahore and of non-diseased healthy individuals from general population. Written informed consent was obtained from parents of all participants and was also asked to fill questionnaire to obtain information on history of verbal and nonverbal communication, aggression, social interaction, fluctuation in mood and interest in physical activities. Immunoglobulin (IgG, IgM and IgA) and CRP levels were measured in serum of patients and controls. CRP levels were measured by semi quantitative agglutination method by using CRP Latex kit purchased from CRP: Fortress diagnostics, UK. Immunoglobulin levels were measured by immunoturbidity technique using Kit purchased from Human Diagnostics, Wiesbaden, Germany, run on Micro lab 300 chemistry analyzer manufactured by Merck. All collected information was entered in to SPSS version 20.0 and analyzed through its statistical package. Age was presented in terms of mean and standard deviation while gender was expressed as frequency and percentages. Student t test was applied between autistic patients and control group to compare the means of quantitative variables. A p-value of  $\leq 0.05$  was considered as statistically significant with confidence interval of 95%. Chi-square was used to compare the categorical variables between patient and control groups.

## RESULTS

A total of 50 individuals with 25 cases from the Rising Sun Institute Lahore and 25 controls from general population were taken. Age ranged from 2 to 15 years with mean age of  $9.88 \pm SD$  of  $\pm 3.37$  years. There were 7 (14%) female and 43males (86%) in study population.

In the present study serum CRP levels were raised in patient group as compared to control group and

**Table 1:** Serum CRP levels in autistic patients and their healthy counterparts.

	CRP Levels		Total	P-value
	Negative (6)	Positive (6,12,24,48)		
Cases	15 (60%)	10 (40%)	25	0.000
Controls	25 (100%)	0 (0%)	25	
Total	40	10	50	

p-value was determined by chi square.

p-value

C-reactive protein

were elevated in 10 out of 25 autistic patients while normal in all control individuals. Statistically significant difference was found in CRP levels between these two groups with p-value of 0.00 (Table 1).

Statistical analysis showed that mean IgA level was 184.5, mean IgM level was 214.9 and mean IgG level was 1263 in patient group while 178.6, 221.4 and 1565 was in control group respectively. Statistically significant difference in mean IgG levels was found between case and control group with p-value of 0.03 while no statistically significant difference in IgA and IgM mean levels was found between two groups with p-value of 0.788 and 0.809 respectively (Table 2).

**Table 2:** Serum levels of IgA, IgG and IgM levels in autistic patients and their healthy counterparts.

Immunoglobulin Levels		Mean	Standard Deviation	P-value
IgA levels	Cases	184.5	99.7	.788
	Controls	178.6	40.6	
IgG levels	Cases	1263	490.2	.034
	Controls	1565	488.4	
IgM levels	Cases	214.9	95.1	.809
	Controls	221.4	94.1	

p-value was measured by using student t test.

p-value

immunoglobulin A

immunoglobulin G

immunoglobulin M

**DISCUSSION**

Autism is a complex neurodevelopmental disorder which is characterized by stereotypic behaviors, variable deficits in language, social skills and a wide range of other behavioral problems.<sup>1</sup>As role of immune system is very important in pathogenesis of autism, immune response changes (both innate and adaptive), auto-antibodies against various tissues and involvement of MHC antigens are observed in autistic patients.<sup>7</sup>

Although males are more affected with autism as compared to females but exact cause of this gender difference is not known. According to the present study males were more in number having autism than females with male: female ratio of 6.1:1. This finding was supported by the study of Keller and Ruta<sup>16</sup> according to which males are more in number to develop autism as compared to female with male: female ratio of 4:1.

As inflammation has well documented role in autism according to our study significant difference in CRP levels were found between case and control groups. Similarly, Khakhzad, et al,<sup>11</sup> also suggested that

inflammatory process can play key role in the pathophysiology of autism. They measured the CRP levels in control and autistic patients and mean concentration of CRP in autistic children was significantly higher than control group. The results of their study are supportive with the finding of this research.

As CRP has important role in pathogenesis of autism and elevated level of CRP impair the neurodevelopment of fetus leading to autism. This theory was supported by a cohort study conducted by Brown, et al,<sup>17</sup> to find any association between early gestational maternal CRP levels and risk of development of childhood autism. Results of their study showed that increasing maternal CRP level, were significantly associated with autism in offspring. Moreover, for maternal CRP levels in the highest quintile, compared with the lowest quintile, there was a significant, 43% elevated risk. This finding suggests that maternal inflammation may have a significant role in autism, and other neurodevelopmental disorders.

The most common immune abnormalities observed in children with autism are immunoglobulin abnormalities. It has been reported that more aberrant behaviors are associated with decreased total serum immunoglobulins level.<sup>12</sup> According to the results of our study mean IgG level were low in autistic children than in control group but no significant difference between mean IgA and mean IgM levels were present between these two groups. These findings are supported from a study conducted by Grether, et al,<sup>18</sup> who determine the levels of serum immunoglobulins and CRP in autistic patients and control population. According to this study serum IgG levels were significantly lower in autistic children as compared to controls but no significant difference in IgA and IgM levels was present between two groups. The findings of this research are consistent with our study. Similarly El-Aziz and El-Din,<sup>19</sup> conducted a study to compare the immunoglobulin deficiencies in autistic children and healthy controls. There was significant difference in immunoglobulin levels between two groups. However, in contrast to our study showing raised serum CRP levels in autistic children in this study CRP levels were not significantly different between two groups.

In another study by Heuer, et al,<sup>20</sup> who measured the plasma immunoglobulin levels in autistic patients and control group they found that autistic patients have significantly reduced levels of plasma IgG and IgM as compared to controls. This suggests an underlying defect in immune function contributing in the development of autism. These findings are also consistent with findings of our study. They also observed that decreased serum immunoglobulin levels correlates with behavioral severity. With the progressive decrease in serum immunoglobulin levels there is increase in the severity of autism. A study was also conducted by Gupta, et al,<sup>7</sup> to know the role of immune system in

development of autism. They measured the immunoglobulin levels in autistic patients and healthy controls. By comparing their levels, they observed that autistic patients have significantly reduced immunoglobulins level than controls which support the results of this research.

From this study, it can be **concluded** that serum CRP levels are significantly increased and among immunoglobulins serum IgG levels are decreased in autistic patients as compared to control group. This study may help in better understanding of the role of immune dysfunction in pathogenesis of autism and to develop therapeutic measures to boost the immune system of autistic patient which may help to reduce the severity of disease.

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