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Evaluation of Serum Immunoglobulins (IgM, IgG, IgA) Levels in Children with Autism Spectrum Disorder in Gorgan, Iran

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Abstract

Background

The etiology of Autism Spectrum Disorder (ASD) is still unknown. New evidence is increasing for the involvement of altered immune responses in the pathogenesis of ASD. This study aimed to compare the serum immunoglobulin levels in children with ASD and a group of healthy children.

Materials and Methods: This case-control study was performed on 42 patients referred to the Psychiatric clinics of Taleghani Hospital, (a referral center hospital in Gorgan, Iran), in 2019-2020. After obtaining the informed consent of the patients' parents or guardians, and applying the inclusion criteria and exclusion criteria, children were divided into two subgroups with and without autism according to the Modified Checklist for Autism in Toddlers (M-CHAT). After obtaining informed written consent, 5 ml of the blood sample was taken from each patient for laboratory evaluation of serum Immunoglobulins (IgM, IgG, IgA) levels; then the patients' information (Demographic and laboratory) was recorded in a checklist. Finally, the data analyzed using SPSS software version 18.0.

Results

The results of the study showed that among 42 children participating, 21 were healthy (57.15% male) and 21 had autism (61.90% male). There was no significant difference in the levels of immunoglobulins M and G between the autism and control groups. The serum level IgA in male in the control and autism groups was different ($p = 0.001$), showing low IgA levels in male children with autism.

Conclusion

This study showed the serum level of immunoglobulin A in patients with autism and in males was lower than in healthy children, which may be due to autoimmune disorders, immune system defects.

Key Words: Autism Spectrum Disorder, Children, Immunoglobulin.

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1- INTRODUCTION

Autism spectrum disorder (ASD) refers to a range of conditions characterised by some degree of impaired social behaviour, communication and language, and a narrow range of interests and activities that are both unique to the individual and carried out repetitively. It is estimated that worldwide one in 160 children has an ASD. This estimate represents an average figure, and reported prevalence varies substantially across studies (1). It is a serious social problem and an increasing global burden with implications for public health services (2).

ASD is thought to be a complex disorder with multiple genetic and environmental factors (3). Although many factors associated with the pathogenesis of ASD, including genetic, neurological, environmental, and immune factors, are known, the etiology of ASD has not been well understood, yet and its pathogenesis is still unknown (4). There is growing evidence that the role of the immune system in neuropsychiatric disorders, including ASD, has long been established, and that the immune system plays an important role in nerve development, including regulating neuronal proliferation, synapse formation, and the elimination of apoptotic nerves (5).

Immune system disorders in children with autism include increased self-efficacy and decreased immune function (6). In 1976, a study found that 5 of 13 autistic children had undetectable antibody titers despite previous vaccination against Rubella while every control subject had detectable titers, making the first suggestion of a link between the immune system and ASD (7, 8). One of the first clues concerning lymphocyte pathology in ASD was described by Stubbs and Crawford who found decreased lymphocyte response to stimulation with Phytohaemagglutinin (PHA) in children with ASD (9). Serum immunoglobulin A (IgA) deficiency was found in 40 individuals with ASD, both children and adults. Eight of 40 studied ASD patients had IgA levels below normal range adjusted for age, while in control groups there were no abnormalities (10). Analysis of plasma levels of immunoglobulins in over 100 individuals with ASD revealed reduced levels of IgG and immunoglobulin M (IgM) that inversely correlated with scores on the Aberrant Behavior Checklist (ABC), with lethargy being especially pronounced in children with the lowest IgG (11). In another study, Plasma concentration of IgM as well as IgG, especially IgG4, was reported to be increased in ASD patients in comparison to healthy siblings. Moreover, IgG1 subclass was found to be increased in comparison with healthy siblings of the same gender (12).

Severity of ASD, measured with Childhood Autism Rating Scale (CARS), was found to be correlated with serum anti-neuronal (13), and anti-ganglioside M1 antibodies (14). Anti-brain antibodies have been found to correlate with more impaired cognitive functions, motor stereotypies (15) irritability, and lower expressive language skills (16). ASD is very complex and heterogeneous. The question of whether immune dysregulation is a primary cause or secondary consequence is still open. Even if immune system integrity turns out to be a key player in ASD pathogenesis, it surely will not be the sole factor responsible for behavioral abnormalities.

However, evidence for an immunological component is strong. Taken together, the presented data suggest a strong link between autism and immune dysfunction. The association between immune system dysfunction and behavioral abnormalities, in at least a subset of individuals with ASD, suggests a potential role for immunomodulatory therapies as a causative treatment (17, 18).

However, despite careful efforts, the pathology of ASD are still unknown and there are currently no biological markers for all people with ASD. Therefore, we intended to conduct a study in order to investigate the relationship between the levels of serum immunoglobulins and autism in sample patients with a definitive diagnosis of autism referred to Taleghani Hospital in Gorgan, Iran.

2- MATERIALS AND METHODS

2-1. Study design and population

Immunoglobulin g are part of the humoral immune response, the net result of a specific response orchestrated by the complex interaction between dendritic cells, T cells, and Ig-producing B cells. Ig levels are therefore a means to measure not only immune development but successful humoral immune function as well. Ig are of particular interest in childhood disorders because levels are very low at birth and it may take up to 10 years for certain isotypes to reach adult levels. Here in, we describe decreased levels of IgG and IgM in children with autism. In addition, we analyzed the relationship between plasma levels of IgG and IgM and behavior (19). This case-control study was performed as a simple sampling on children referred to specialized children's psychiatric and asthma and allergy clinics of Taleghani Hospital (referral center), Gorgan, Iran, in 2019-2020 during 8 months.

2-2. Inclusion criteria

- 3-12 years old.
- Absence of other psychiatric and physical disorders in ASD children.
- Parents' consent to participate.

2-3. Methods

After obtaining the informed consent of the patients' parents or guardians, and applying the inclusion criteria and exclusion criteria, children were divided into two subgroups with and without autism according to the Modified Checklist for Autism in Toddlers (M-CHAT). Also, the control group was selected from patients referred to pediatric asthma and allergy clinic who did not have any psychiatric symptoms. The subjects in the control group were matched with the case group (ASD) in terms of gender and age. 5 ml of the brachial vein blood sample was taken from each participant and used to estimate the serum level of immunoglobulins (IgA, IgM, and IgG) in children. A checklist was prepared and recorded for each participant including demographic information (age, gender, parents' education, place of residence, and type of housing), and serum immunoglobulin levels.

2-4. Laboratory measurements

2-4-1. Plasma Collection

5 milliliters of blood from each child was collected in yellow top citrate tubes according to the study protocol and centrifuged at 900g for 10 min to pellet cells. Plasma was collected and immediately frozen in 0.5mL aliquots at −80°C until assayed for Ig levels.

2-4-2. ELISA

Levels of total IgG, IgM, IgA, and IgE were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits purchased from ALerCHEK Inc. Kits were run according to the manufacturer’s instructions. Briefly, samples were diluted 1:100,000 (IgG), 1:10,000 (IgM and IgA) (**Table.1**). After 1-hr incubation and subsequent washing, horseradish peroxidase- conjugated detection antibodies were added and tetramethyl benzidine/peroxide substrate used for development. Data are reported as median mg/mL (IgG, IgM, and IgA) (**Table.2**). Immunoglobulin values of each patient were compared with standard normal values according to his/ her age.

Table-1: Standard normal values of Immunoglobulins (IgG, IgA, and IgM).

Age	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)
< 1year	172-1069	41-173	11-106
1-5 years	345-1236	43-207	14-159
6-10 years	608-1572	52-242	33-236

Ig A: Immunoglobulin A.

Table-2: Standard normal values of Immunoglobulins (IgG2 and IgG4).

Age	IgG4	IgG2
<5 months	≤19.8	≤82
5-9 months	≤20.8	≤89
9-15 months	≤23	24-98
15-24 months	0.4-49.1	35-105
2-4 years	0.8-81.9	39-176
4-7 years	1.0-108.7	44-316
7-10 years	1.0-121.9	54-435

Ig G: Immunoglobulin G.

2-4-3. M-CHAT

In the original validation study with 1293 children, M-CHAT has a sensitivity of 0.87, specificity of 0.99 but a low positive predictive value of 0.36 although this rose to 0.68 when combined with a follow-up interview to clarify parental understanding of the items and the child's behaviours In a further study with 3793 children, the positive predictive value was 0.74 although most of those assessed came from the sample of children referred because they were perceived to be of high risk for autism (20, 21) .However Robins in a study of unselected toddlers reported a positive predictive value of 6 % but this rose to 57 % after follow-up interviews (22), In these studies the internal reliability of the M-CHAT was high with a Cronbach's alpha of 0.85. International studies of M-CHAT have broadly replicated these findings (23).

2-5. Ethical considerations

The Declaration of Helsinki was adhered to set the ethical principles of this study that was approved by the Ethics Committee of Gorgan University of Medical Sciences, Iran (IR.Goums.REC.1398.346). In addition, all participants signed informed written consent to participate in the intervention. This article has been adapted from the Medical doctor thesis written by Shoeib Safaei in the internal medicine department of the Gorgan University of Medical Sciences, Iran.

2-6. Data analyses

Finally, the data were entered into SPSS version 18.0 and described through measures of central tendency and dispersion (mean, median, standard deviation), as well as tables and graphs. Shapiro-Wilk test was used to analyze the mean serum immunoglobulin level in the groups. In the case of normality, independent t-test, and in the absence of normality assumptions, the Mann-Whitney U test was used. The significance level was set at 0.05.

3- RESULTS

3-1. Demographic variables

In this study, out of 42 participated children, the number of two groups was equal (21 healthy and 21 ASD). The mean age of all children was 6.21 ± 2.49 and 24 (57.15%) male and 18 (42.58%) female. Also, in the study of the education level, it

was found that among the fathers participating in the study and among mothers, 30 (71.42%), and 8 (38.10%) had a university education. The observed differences in terms of the father's (P = 1.000), and mothers' (P = 0.217) education level between the autism and control groups were not statistically significant (**Table.3**). In this study, participants were asked about their area of residence (urban-rural) type of house (house-apartment). According on **Table.3**, there was no significant difference between the area of residence (P = 0.317), and of type of house (P = 0.095) the two groups.

Table-3: Comparison of the mean and standard deviation of demographic characteristics in two groups of autism and control.

Participants	Number (%)	Sub-group	Total	Control group	ASD group	*P-value
		Total	42	21	21	-
		Male	24 (57.15%)	12 (57.15%)	12 (57.15%)	
		Female	18 (42.85%)	9 (42.85%)	9 (42.85%)	
Age, year	Mean± SD	Total	6.21±2.49	6.28±2.43	6.14±2.61	0.751
Type of house	Number (%)	House	13 (30.95%)	9 (42.85%)	4 (19.05%)	0.095
		Apartment	29 (69.05%)	12 (57.15%)	17 (80.95%)	
Region	Number (%)	Urban	29 (69.05%)	13 (61.90%)	16 (76.19%)	0.317
		Rural	13 (30.95%)	8 (38.10%)	5 (23.81%)	
Father's level of education	Number (%)	None university education (≤ diploma)	12 (28.58%)	6 (28.57%)	6 (28.57%)	1.000
		University education	30 (71.42%)	15 (71.42%)	15 (71.42%)	
Mother's level of education	Number (%)	None university education (≤ diploma)	22 (52.38%)	13 (61.90%)	9 (42.85%)	0.217
		University education	20 (47.62%)	8 (38.10%)	12 (57.15%)	
*Chi-Square test, SD: Standard deviation.						

The difference in the mean serum IgM and IgG levels in children with and without autism were not significant (P= 0.431, P= 0.850). Also, Results showed that there was no significant difference in the level of immunoglobulin M and G with gender between the control and autism groups (P> 0.05). The mean serum IgA levels in the healthy and autism groups were 0.43 ± 2.02 and 1.54± 0.63, respectively, which was significant (P = 0.012), and indicated a lower serum IgA level in children with autism. Moreover, the IgA level was assessed according gender and showed significant difference in male (P = 0.001). In experiments performed on children participating in the study, the mean serum level of IgG2 in all participants, as well as in the control and autism groups are listed in the table below, showing that the difference observed in the groups was not statistically significant (P = 0.890). The IgG2 level in patients was also examined by gender and showed that there was no statistically significant difference in the IgG2 level between the two groups (P >0.05). In the present study, the mean serum level of IgG4 was 641.33 ± 182.53 and it was not significantly different between the two study groups. The IgG4 levels by gender in male and female children in the control and autism groups showed no statistically significant difference (**Table.4**).

Table-4: Comparison of the mean of serum immunoglobulin levels in the two groups of healthy children and children with autism.

Immunoglobulin level	Sub-group		Control	ASD	*P-value
IgM (mg/dl)	Mean± SD	Total	86.66±33.12	81.40±36.37	0.431
		Male	86.50±37.34	82.76±39.14	0.650
		Female	86.88±28.73	79.20±33.85	0.620
	Minimum		52.00	41.00	
	Maximum		170.00	162.00	
IgA (mg/dl)	Mean± SD	Total	2.02±0.43	1.54±0.63	0.012
		Male	2.06±0.39	1.35±0.53	0.001
		Female	1.96±0.50	1.86±0.69	0.722
	Minimum		0.80	0.64	
	Maximum		2.50	2.80	
IgG (mg/dl)	Mean± SD	Total	13.00±3.73	13.20±3.58	0.850
		Male	11.95±2.19	12.74±4.01	0.554
		Female	14.38±4.94	13.95±2.85	0.829
	Minimum		8.50	8.60	
	Maximum		23.00	22.50	
*Mann-Whitney Statistical Test					**P-value
IgG2 (mg/dl)	Mean± SD	Total	1209.85±440.89	1281.33±593.51	0.890
		Male	1048.83±269.61	1331.69±663.76	0.247
		Female	1424.55±543.38	1199.50±488.67	0.386
	Minimum		750.00	730.00	
	Maximum		2500.00	3028.00	
IgG4 (mg/dl)	Mean	Total	637.23±183.69	645.42±185.81	0.886
		Male	634.33±126.96	618.07±185.73	0.802
		Female	641.11±249.32	689.87±189.38	0.660
	Minimum		220.00	396.00	
	Maximum		980.00	953.00	
**T-test, SD: Standard deviation.					

4- DISCUSSION

In this study, patients referred to children's neurology and psychiatric clinics with ASD diagnosis were selected as the autism group, and children with no ASD who referred to children's asthma and allergy clinic were assigned into the control group. The mean serum IgM levels in children with and without autism were 81.40 ± 36.37 and 86.66 ± 33.12, respectively, but the difference between the groups was not statistically significant (P=0.431). These findings are consistent with the studies conducted by Chaudhry et al. (p=0.809), (24), Spiroski et al. (p=0.268), (25), Croonenberghs et al. (p=0.97), (26), and Wasilewska et al. (p>0.05), (6); but, Heuer et al. (11), designed a study to assess the level of immunoglobulin in children with autism or developmental delays compare from those with typical development. Analysis of plasma levels of immunoglobulins in over 100 individuals with ASD revealed reduced levels of IgG and immunoglobulin M (IgM) that inversely correlated with scores on the Aberrant Behavior Checklist

(ABC), with lethargy being especially pronounced in children with the lowest IgG. According to various similar studies, as well as the appropriate sample size in each of the studies, there does not appear to be a difference in serum immunoglobulin M levels in patients with autism compared to healthy children. In this study, the mean serum IgG levels in the control and autism groups were 13.00 ± 3.73 and 13.20 ± 3.58, respectively (p=0.850). This finding was similar to the study of Spiroski et al. (p=0.359), (25), which showed no difference in serum IgG levels between the two groups, while in the study of Croonenberghs et al. (p=0.023), (26), Chaudhry et al. (p=0.034), (24), the findings indicated an increase in serum IgG levels in children with autism. Some differences in the significance of immunoglobulin levels in children with autism compared to healthy individuals may be due to age. Age is thought to be an influential factor that changes IgG levels significantly and rapidly during the first decade of life.

In this study, the serum level of the IgG2 subgroup was also examined, which was 1209.85 ± 440.89 and 1281.33 ± 593.51 in the control and autism groups, respectively (p = 0.890). These findings are similar to the study by Grether et al. (p=0.251), (27), which showed no significant difference between healthy children and those with autism, while in the study of Croonenberghs et al. (p=0.009), (26), the findings showed an increase in serum IgG2 levels in children with autism. Results vary and are often contradictory, these inconsistencies may be due to small sample sizes and improper controls such as "population standard" versus age-matched controls residing in the same locale, and lack of adjusting for seasonality. In addition, as a highly heterogeneous disorder, the behavioral phenotype of the subjects studied may also affect the outcome (28).

In this study, Serum immunoglobulin A levels were evaluated among the study children. The mean serum IgA levels in the healthy and autism groups were 0.43 ± 2.02 and 1.54± 0.63, respectively (p=0.012), which indicated a lower serum IgA level in children with autism. Comparing immunoglobulin levels across a broad age range can produce inconsistencies, thus it is critical to have age-matched controls. For example, For example, an early report found decreased circulating IgA associated with HLA-DR antigens in a subset of ASD subjects and a 2012 study supported these findings (6). On the other hand, a study by Zhou et al. (29) on the stool samples in a group of children with autism reported an increase in fecal IgA levels (p<0.001); however, other studies by Chaudhry et al. (p=0.788), (24), showed no change in IgA. In a study on 31 patients with selective IgA deficiency, 1 had a diagnosis of ASD. The researchers focused on the offspring and siblings of the abovementioned group.

Out of 87 children born to individuals with IgA deficiency, 3 had a diagnosis of ASD in comparison to 1 child out of 193 children born to subjects with normal IgA concentration. ASD was diagnosed in 2% of siblings (2/99 individuals) of IgA-deficient patients in contrast with 0.5% of siblings (1/217 individuals) in the control group. However, the abovementioned results did not reach statistical significance (30). Differences in Immunodetection technique would also have the potential to introduce variability between studies. Another immunoglobulin assessed in this study was IgG4, the mean of which was not significantly different between the control and autism groups (p= 0.886). This finding is inconsistent with the findings of similar studies including those conducted by Zacky et al. (p<0.05), and Enstrom et al. (p=0.01); because, in these studies, IgG4 was introduceda relatively unique subclass of IgG. IgG4 unlike IgG2 does

not bind strongly to any of the antibody receptors (CD16, CD32) found on human leukocytes. IgG4 binds to the receptor CD64 (FcγRI) on monocytes and macrophages with ten times less affinity than either IgG1 or IgG3. Moreover, the circulating structure of IgG4 is functionally monovalent and differs from IgG2 which contain two binding sites. These features drastically alter the biological function of the IgG4 antibody, shifting its function to that of a blocking or inhibiting antibody rather than one of protection through the more conventional routes, such as complement fixation (31, 32).

4-1. Study Limitations

There are several limitations to this current study. First, while we did not include any participant who showed visible signs of illness or who had a fever, our use of a single cross-sectional obtained blood sample would not capture temporal fluctuations in the IgG isotype levels based on health status or environmental factors such as vaccinations and socioeconomic and demographic factors.

5- CONCLUSION

In this study, there was no difference in serum levels of immunoglobulins between case and control groups and in both gender. While, Serum immunoglobulin A levels are lower in patients with autism and in males than in healthy children .This decrease in concentration may be due to autoimmune disorders, immune system defects, or genetic defects in these children, which indicates the need for further investigation.

6- CONFLICT OF INTEREST: None.

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