Original Article

Effect of Gender on Salivary Immunoglobulin and Complements in Autism Children

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Abstract

Background: Autistic spectrum disorder (ASD) is considered a disease of neurological development; until now, there are no specific tests or biological indicators that may be utilized to establish the diagnosis. A delay in ASD diagnosis delays the initiation of pharmacological and therapeutic interventions, preventing biopsychosocial development and lowering the individual's prognosis. **Objectives:** This study aimed to find a correlation between ASD and gender effect for some salivary immunoglobulin (Ig) and complements to facilitate the diagnosis ASD. **Materials and Methods:** It involved 30 child diagnosed with autism, aged 6- to 12-year-old. Those children were represented all ASD children of both sexes who satisfied the criteria of diagnosis for autism, saliva samples were collected and enzyme-linked immunosorbent assay kit was used to measure C3, C4, IgA, and IgG. **Results:** The result shows a significant increase in the concentration of IgG in male children as compared to female children, in spite of higher level of the rest variables for the male children with ASD. **Conclusion:** There was an effect of gender on salivary Ig in patient with ASD.

Keywords: Autistic spectrum disorder, complements, salivary immunoglobulin

Introduction

Autistic spectrum disorder (ASD) is a disease of neurological development identified by a deficit in personal ability to communicate, to socially interact, and to learn, as well as restricted and repetitive type of attitude, like continuous motions, unchanged attention, and the way they respond to sensory stimuli by over or under reactivity.[1] These qualities differ in the style and severity with which they exhibit themselves in more than one person in a very specific way, and they affect how these persons relate, behave, and express themselves. The prevalence has been steadily increasing over the past two decades, with current estimates reaching up to 1 in 36 children.[2,3] According to the World Health Organization, ASD affects one out of every 160 children, with boys being affected four times more than girls. ASD is more common in Caucasians than in Afro-descendant and hispanic children. ASD is a heterogeneous behavioral disorder that is characterized by qualitative deficits in social communication and interaction and restricted, repetitive behavioral patterns, activities, and interests.[3,4]

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A considerable awareness and an elevation in the diagnosis of ASD has been detected in recent decades, making it a major public health concern.[5] Diagnosis of ASD is a challenging and sophisticated procedure, and it is performed by a multidisciplinary team based on clinical observation and investigation of the child's behavior. Anyhow, there are no specific tests or biological indicators that may be utilized to establish the diagnosis.[6] A delay in ASD diagnosis delays the initiation of pharmacological and therapeutic interventions, preventing biopsychosocial development, and lowering the individual's prognosis.[7]

ASD is a multifactorial disease, according to the literature (genetic, immunological environmental, inflammatory, and metabolic factors) seem to take part in the disease process. The first 5 years of a child's life is a golden

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period for their growth and development, fostering their future learning skills and social and emotional abilities due to rapid gains in physical and cognitive growth and development.[8,9] Saliva is a biological fluid with a variety of activities, including oral mucosa preservation and cleansing, as well as antibacterial and digesting properties.[10,11]

Immunoglobulins (Ig) are a component of the humoral immune response, which is the consequence of a complicated interaction involving T cells, dendritic cells, and Ig-producing B cells. As a result, levels of Ig can be used to assess not just the evolution of immune system but also successful activity of humoral immune system. Because Ig are at a minimum concentration at birth and it may need to 10 years for specific isotypes to peak up to mature person levels, Ig are of special relevance in juvenile diseases.[12] In saliva, IgA is the most common Ig isotype.[13] In oral mucosal immunity, salivary IgA is the most important defensive antibody. Women and children are among the most vulnerable in times of disaster. IgA is the most prevalent immunoglobulin isotype in saliva.[7] Salivary IgA is the most significant defense antibody in oral mucosal immunology. Children and women are among the most vulnerable groups during disasters, and they require regular but crucial care.[8,9,14,15]

Immunity of oral mucosa is primarily protected by salivary IgA.[16] In people with ASD, salivary IgA levels, which are regulated by oral microorganisms, may fluctuate. In saliva, the most prevalent Ig isotype is IgA. Within the salivary glands stroma, IgA is generated by specific local plasma cells and conveyed to the mouth by the receptor of polymeric immunoglobulin (PIGR) on monomeric IgA and salivary gland ductal cells, which is obtained from serum itself.[13] sIgA is the most important defensive antibody involved in the immunity of oral mucosa, working in tandem with the innate immune system to prevent attachment of microbes on both mucosal surface and dental surface, allowing cariogenic bacteria such as *Streptococcus mutans* to be eliminated.[14,17] Patients with a lack of salivary IgA are more susceptible to infections of oral mucosa and tooth caries.[18,19]

The immune system is composed of the two types namely adaptive and the innate immune systems. Complement system is one of the innate immune systems' key effector systems, signaling for greater inflammation and clearing pathogens and cell debris. Complement system is made up of a variety of inert components that are activated in a series-like procedure in the innate immune system to exert biological effects. Three major mechanisms can start the complement cascade: the classical pathway, the lectin pathway, and the alternative pathway.[20,21] There are two types of immune systems: adaptive and innate. The complement system is one of the innate immune systems' key effector systems, signaling increased inflammation and clearing pathogens and cell debris. The complement system is made up of a variety of inert ingredients that are activated in a cascade-like fashion in the innate immune system to exercise its biological effects. There are three major mechanisms that can start the complement cascade: classical, lectin, and alternative.[20,22,23] On the cell surface, Mannose-binding lectin (MBL) binds to mannose residues, which initiates the lectin cascade.[20] This stimulates the MBL-associated proteases MBL serine protease 1 (MASP1) and MASP2, which split C4 to yield C4b2b, a C4 convertase.[21] The alternative pathway is activated by a "tickover" mechanism (through C3 hydrolysis), that acts as a C3b amplification loop after the classical and lectin pathways have been initiated.[21] All three mechanisms lead to the activation of C3, the primary complement component. This activates microglia, causing them to emit inflammatory signals, as well as the process of pathogens phagocytosis and T cells, which produce cytokines such as interleukins.[20,22]

Materials and Methods

This research took place from October through December of 2021. It involved 30 children diagnosed with autism, aged 6–12 year-old. Those children were represented all ASD children of both sexes who satisfied the criteria of diagnosis for autism as established in the Universal Diseases classification, edition 10 during the study period (The International Classification of Diseases, Tenth Edition [ICD-10]) (repetitive, limited, and stereotyped style of attitude, activities and interests, as well as qualitative anomalies in communication, reciprocal social contact, and limited, recurrent, stereotyped manner of behavior, activities and interests).[24] The study excluded children with autoimmune diseases (autoimmune blood disease, systemic lupus erythematosus (SLE), and rheumatoid arthritis) and allergic diseases (asthmatic patients, allergy of skin, and allergic rhinitis).

After explaining the study's purpose to the parents, they signed a written informed consent form. All the patients' information was kept private, and neither the information nor the samples gathered were used in any other study. The National Research Centre's ethical committee gave their approval to perform this study. Enzyme-Linked Immunosorbent Assay kit is used to determine the amounts of complement C3 in human saliva. A complement C3 specific antibody was pre-coated and blocked onto 96-well plates of ELISA. After adding standard or test samples to the 96 wells, a complement C3 specific biotinylated detection antibody is added, and the wells are washed with wash buffer. Wash buffer was used to wash away the unbounded conjugates after Streptavidin-Peroxidase Conjugate is applied. The Streptavidin-Peroxidase enzymatic reaction is then seen using tetramethylbenzidine (TMB), which is catalyzed by

Table 1: The descriptive statistics including (number of the patients, minimum and maximum value, mean, and standard deviation) for each variable in male autistic children

Variables in autism children (male)	*N*	Minimum	Maximum	Mean	SD
C3	15	0.87	1.53	1.1720	0.19839
C4	15	0.30	0.68	0.4787	0.12176
IgA	15	0.66	2.00	1.6373	0.34323
IgG	15	1.00	1.80	1.5400	0.30892

Table 2: Mean and standard deviation for all variables in female autistic children in the study)

Variables in autism children (female)	*N*	Minimum	Maximum	Mean	SD
C3	15	0.79	1.24	0.9700	0.22819
C4	15	0.30	0.50	0.4307	0.07815
IgA	15	1.02	1.96	1.3960	0.47667
IgG	15	1.00	1.20	1.0800	0.10142

Streptavidin-Peroxidase, producing a blue product which turns into yellow at the time the acidic stop solution is added. The collected amount of complement C3 on plate is directly related to the density of yellow color.[25,26]

In order to measure the amount of complement C4 in saliva; we utilize the same procedure by ELISA.[27,28]

In order to clarify amount of a target which between two antibodies, the human IgA solid-phase sandwich ELISA test is performed. In the wells of the supplied microplate, a target-specific antibody has been pre-coated. Thereafter, these wells are loaded with test samples, standards, or controls, which bind to the immobilized antibody. The second antibody is added to the sandwich, followed by a substrate solution that reacts with the enzyme-antibody-target complex to produce a quantifiable signal. The strength of this signal is proportional to the amount of target contained in the original specimen.[29,30]

The same procedure is performed; ELISA human IgG solid-phase sandwich technique is utilized to identify the magnitude by which a target of two antibodies has been bounded between them in the saliva.[31,32]

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 342 (including the number and the date in 2021/6/13) to get this approval.

Results

Table 1 shows the descriptive statistic of the present study because the means of C3, C4, IgA, and IgG among the male autistic children were 1.1720 mg/dL, 0.4787 mg/dL, 1.6373 µg/mL, and 1.5400 IU/mL, respectively.

Table 3: Significance difference between male and female (children) for the all variables

	t-test	*df*	Sig.
C3	2.587	27.469	0.15
C4	1.285	23.860	0.211
IgA	1.591	25.442	0.124
IgG	5.479	16.983	0.000

Table 4: Correlation between study variable among autistic female children group

	C4	IgA	IgG
C3	*r* (–0.403) *p* (0.136)	*r* (0.430) *p* (0.109)	*r* (–0.068) *p* (0.810)
C4		*r* (–0.483) *p* (0.068)	*r* (–0.112) *p* (0.690)
IgA			*r* (0.141) *p* (0.616)

While the means of C3, C4, IgA, and IgG among female autistic children were 0.9700 mg/dL, 0.4307 mg/dL, 1.3960 µg/mL, and 1.0800 IU/mL respectively as demonstrated in Table 2.

Table 3 revealed that there are insignificant differences in concentration of C3, C4, and IgA between male and female autistic children, while there is highly significant difference in level of IgG since its level in saliva of the male is higher than that among the female autistic children.

About the correlation between study variable among autistic male and female children group, the results of the current study revealed that there wasn't any correlation in male children group [Table 4], on the other hand in female children group there was a positive relationship between C3 and both IgA and IgG on one side and there is inverse relationship between C4 and both IgA and IgG on other side. Whereas there is inverse relationship between C3 and C4 as shown in Table 5.

Table 5: Correlation between study variable among autistic male children group

	C4	IgA	IgG
C3	*r* (–0.764)** *p* (0.001)	*r* (1.000)** *p* (0.001)	*r* (1.000)** *p* (0.000)
C4		*r* (–0.764)** *p* (0.001)	*r* (–0.764)** *p* (0.001)
IgA			*r* (1.000)** *p* (0.001)

Discussion

ASD is common neurodevelopmental disorder, it affects males four times more than females (one out of every 68 children in 2014). Comparing it to patients with an acute phase of autism, this ratio is larger in those with a mild phase of the disease.[33] The current study found no statistically difference in salivary C3 and C4 levels between male and female autistic children, and this study agrees with Ashaat *et al*.,[34] who found no statistically significant difference concerning salivary C3 and C4 levels between autistic children and the healthy individuals. They also showed statistically no worthy difference in levels of CD4, C3, and C4 in serum among individuals with positive and negative gastrointestinal tract (GIT) symptoms.

Furthermore, the present study demonstrated that there is a highly significant difference in IgG levels, with male autistic children's saliva being higher than female autistic children. This research aligns with a 2008 study by Heuer *et al*.,[35] who discovered that children with autism have a considerably descend amount of plasma IgG than healthy children, and they attributed the cause to an underlying immunosuppression status. A decrease in levels of specific Ig is related to intensity of behavior, so that, patients with the highest behavioral battery scores having less amount of IgG levels.

This study demonstrated no significant difference in salivary IgA levels between male and female autistic children, which contradicts a previous study by Gong *et al*.,[36] which reported that salivary IgA level was considerably in low amount in children with ASD than in healthy control group. Another study established in Venezuela investigated the propensity in salivary IgA content drop among ASD children and healthy individuals, (number; 34 for each group) who aged (4–13) year-old, but no statistical significance was found. This could be due to the diurnal rhythmicity of human salivary IgA level causing data variance,[37] because the author did not specify if the collection of saliva procedures were all done at the same time. In oral cavity, the fundamental protective antibody is salivary IgA. It suppresses the adhesion of the bacteria to the surfaces of epithelial and tooth, lowers the dental plaque buildup by acting against Streptococci glucosyl-transferases, and participate with salivary innate immune system.[38] For that reason, a reduction in the levels of IgA may negatively affect the harmony status of oral health.

Conclusion

There was effect of gender on salivary Ig in patient with ASD. A significant increase in concentration of IgG in male children as compared to female children, and the presence of higher level of the other Igs for the male children may assist the diagnosis of ASD.

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Conflicts of interest

There are no conflicts of interest.

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