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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2024.487.492

Research Article
Investigating the Role of Gluten Sensitivity in the Etiology of Autism Spectrum Disorder

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Abstract

Background and Objective: Autism Spectrum Disorder (ASD) is a range of neurodevelopmental disabilities that lack a clear etiology. To date, studies investigating the role of immune reactivity to gluten in ASD have been inconsistent. This study aimed to compare levels of gluten reactivity markers in 319 ASD patients to 172 of their unaffected siblings and 322 of unrelated healthy controls (UHC). **Materials and Methods:** Patients younger than 12 years old diagnosed with ASD via experienced child psychiatrists and neuro-pediatricians were recruited and gluten reactivity markers and gastrointestinal (GI) complaints commonly found in children with ASD were also investigated. Serum levels of anti-gluten and anti-tissue transglutaminase IgA (anti-TTG IgA) were measured via ELISA. **Results:** No significant differences were detected in IgA levels and IgG levels for anti-TTG among all groups ($p<0.05$). The anti-gliadin IgG levels were significantly higher in ASD patients and their siblings compared to the UHC group ($p<0.05$). Also, those IgG levels were not associated with any GI complaints or Electroencephalogram (EEG) abnormalities ($p>0.05$). **Conclusion:** The data suggested gluten sensitivity has no role in ASD pathophysiology or its comorbid symptoms.

Key words: Autism, celiac disease, gliadin, gluten sensitivity, gut-brain axis, brain, autism spectrum disorder

Citation: AL-Dalaeen, A., N. Abu Tarboush, L. Alzghoul, A. Al-Akily, E. Almaqtari, E. Abdo and S. Alsadi, 2024. Investigating the role of gluten sensitivity in the etiology of autism spectrum disorder. Pak. J. Biol. Sci., 27: 487-492.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The complex neurodevelopmental disorder known as autism spectrum disorder (ASD) is characterized by several symptoms that usually manifest before the age of three. These symptoms include a persistent deficit in social communication and interactions, stereotypical behaviors and limited verbal and nonverbal communication[1]. Among all mental diseases, ASD is thought to be the primary cause of disability in children under the age of five and the fourth in those between the ages of five to fourteen[2]. The etiology of ASD is complex and still not well understood[3]. However, several studies that investigated the etiology of ASD suggest many factors, such as underlying genetic predisposition and environmental factors, yet, none of these factors approved to have a full association so far and further investigation to understand its pathophysiology is needed[4,5]. In addition to the above-mentioned core symptoms, children with ASD have many comorbidity symptoms, such as abnormal EEG and gastrointestinal (GI) complaints. Recent studies demonstrated that almost 70% of children with autism develop GI symptoms, which is 5 to 6 times higher than the normal population, these complaints are usually frequent constipation, persistent diarrhea, as well as bloating and pain[6]. Studies found that these complaints may vary widely in their nature between patients[7], besides, their existence and severity are positively associated with the severity of the disease[8]. For instance; studies revealed that patients with GI problems have more severe symptoms on measures of irritability, anxiety and social withdrawal[9], as well as, maladaptive behaviors[10]. This possible link may be due to common pathophysiological and pathogenetic factors[7].

In reaction to some foods, the immune system generates immunoglobulin G (IgG) antibodies; these antibodies mix with the food antigen in the circulatory system to form immune complexes that can build up in tissues[11]. These immune complexes cause the body, particularly the brain, to become more inflammatory by releasing pro-inflammatory cytokines[12]. Abnormal intestinal permeability was reported not only in patients with ASD but also in their first-degree relatives[13]. Studies commonly described this higher intestinal permeability and dysfunction of the intestinal barrier as "leaky gut syndrome"[14,15], which was found to be associated with autism It was noticed that this process progresses as long as the intake of gluten continues[14].

The anti-gliadin IgA, anti-gliadin IgG and tissue transglutaminase (tTG) measurements can be used as a first-line serological method to diagnose celiac disease. People with non-celiac gluten sensitivity can also test positive for elevated levels of these immunoglobulins. In an area where this kind of research has not before been done, the current study sought to examine gluten-related immune reactivity in a sample of children diagnosed with ASD without gastrointestinal symptoms. This is noteworthy because variations in ASD epidemiology may also be reflected in the activity of the gut-brain axis. Children with ASD had their levels of anti-tTG, anti-gliadin IgA and anti-gliadin IgG tested and their results were compared to those of healthy children. Therefore, this study aims to investigate the serum levels of markers of gluten-related immune reactivity in children with ASD. Compared to their unaffected siblings and unrelated healthy control children.

MATERIALS AND METHODS

Study area: For this study, siblings and patients under the age of 12 who had been diagnosed with ASD by skilled child psychiatrists and neuro-pediatricians were gathered from pediatric clinics and nearby special healthcare facilities during 2020-2021 in Amman, Jordan. The research team questioned the ASD group after obtaining written agreement from their parents to compile their medical history, arrive at a single diagnosis and ensure that the diagnosis fits both of them.

Subjects: The ASD section of the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-V) as well as Childhood Autism according to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) criteria. Karyotyping was performed for all autistic subjects to exclude syndromic autism and genetic abnormalities that are associated with ASD. Total IgA was also measured to exclude a selective decrease in IgA.

Other exclusion criteria included chronic illnesses, history of known allergies, symptoms of illness and infection currently or in the last 3 months and restricted diet. As a result, a total of 319 ASD patients (245 males and 74 females) and 172 unaffected siblings (121 males and 51 females) were included in this study. As for the controls, they were recruited from visitors to the Jordan University Hospital (JUH) for any reason other than an acute or chronic disease, for example, children having regular checkups and/or immunization shots, and/or children accompanying a family member to the hospital. Exclusion criteria for controls were the same as those for the cases, with the additional exclusion criterion of a family history of ASD. As a result, 327 unrelated healthy controls (UHC) were matched for sex and age and assigned to the UHC group.

Ethical consideration: The study was approved by the research committee at the School of Medicine, The University of Jordan and the Ethics Committee of JUH (reference number 10/2018/1181) and was carried out by The Code of Ethics of the World Medical Association.

Since seizures and abnormality in Electroencephalogram (EEG) records are more frequently reported in ASD children compared to healthy ones and linked to the severity of ASD cases, available EEG records have been classified as "Normal" or "Abnormal" depending on the EEG report if it indicated any abnormality regardless of the nature or severity of the abnormality reported. The GI symptoms for ASD include abdominal pain, constipation and diarrhea[16].

Blood collection and antibody levels measurement: A total of 3 mL of fasting venous blood was collected from each participant into a plain tube. Following centrifugation, serum was transferred to an Eppendorf tube and stored at -80°C until use for antibody level quantification. Serum levels of total IgA, anti gliadin IgA, anti-gliadin IgG, anti-tissue transglutaminase IgA (anti-TTG IgA) and anti-TTG IgG were measured using specific individual ELISA kits for each antibody (MyBioSource, Inc. San Diego, California, USA) following the manufacturer's recommendations. Later, ELISA plates were read by a BioTek plate reader (Vermont, USA) and a graph with antibody levels was drawn using the reader software (Gen5). All samples were measured 3 times each. Samples that gave spurious measurements were omitted from the study. Data were then averaged for each sample and run for statistical analyses.

Statistical analysis: For quantitative differences in serum antibody levels between groups, statistical differences were investigated using a One-way Analysis of Variance (ANOVA) test with Bonferroni *post hoc* pairwise comparisons when needed. To investigate the possible correlation between levels of serum antibodies and GI complaints or EEG abnormalities, a Pearson correlation test was performed. All statistics were performed using the Statistical Package for the Social Science Program (SPSS)-25.0 with a p-value of 0.05 considered to be significant.

RESULTS

Demographics: Descriptive data including gender and age for 319 ASD patients, 172 siblings and 327 UHC were presented in Table 1.

IgA levels and ASD: Levels of anti-gliadin IgA and anti-TTG IgA were measured in ASD children and compared to the levels of their unaffected siblings and UHC. A One-way Analysis of Variance (ANOVA) analysis revealed no significant differences in both IgA levels among the three groups.

IgG level and ASD: Levels of anti-gliadin IgG and anti-TTG IgG were measured in ASD children and compared to the levels of their unaffected siblings and UHC. A One-way Analysis of Variance (ANOVA) analysis revealed no significant difference in anti-TTG IgG levels among the three groups. However, for anti-gliadin IgG, One-way Analysis of Variance (ANOVA) analysis revealed a statistical difference in serum levels between the three groups [$F(2, 815) = 5.411$ and $p = 0.005$]. The pairwise comparison revealed a significantly higher level of anti-gliadin IgG levels in both ASD subjects ($M = 10.33$ and $SD = 11.86$) as well as the siblings ($M = 10.77$ and $SD = 11.04$) compared to the UHC ($M = 7.95$ and $SD = 9.70$) ($p<0.05$). No significant difference was detected between the ASD group and siblings ($p = 1$) as shown in Fig. 1. To investigate if the levels of anti-gliadin IgG are related to the age of the patient, the correlation between levels of IgG and age was investigated using Pearson's correlation coefficient and no significant correlation was detected ($r = -0.073$ and $p = 0.38$) (Fig. 2).

To further investigate the possible role of anti-gliadin IgG levels in the pathophysiology of ASD, the correlation between IgG levels and common comorbidity symptoms

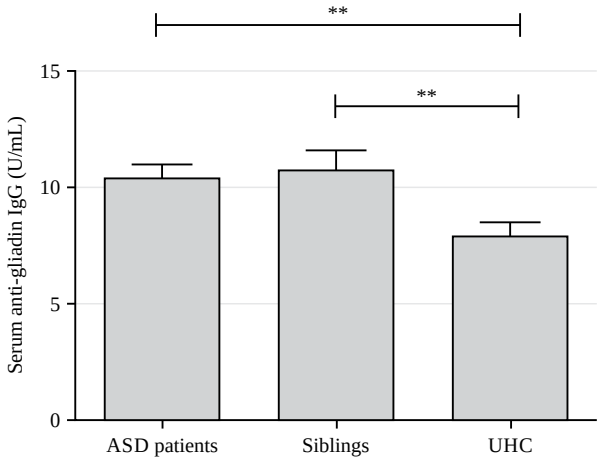

Fig. 1: Anti-gliadin IgG level

Columns represent anti-gliadin IgG levels in autism spectrum disorder (ASD) patients, their siblings and unrelated healthy controls (UHC) groups. The figure shows higher anti-gliadin levels in both ASD and sibling groups compared to UHC, while no significant difference is found between ASD patients and their siblings and **p<0.01

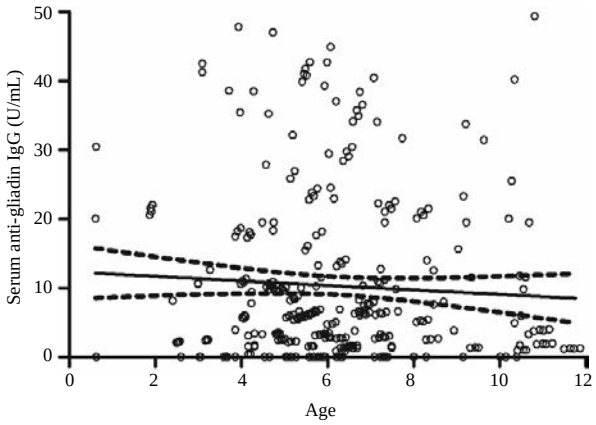

Fig. 2: Serum anti-gliadin IgG levels in relation to age

Scattered plot representation of serum anti-gliadin levels shows no correlation of IgG levels with age in the ASD group and r = -0.06 and p = 0.28

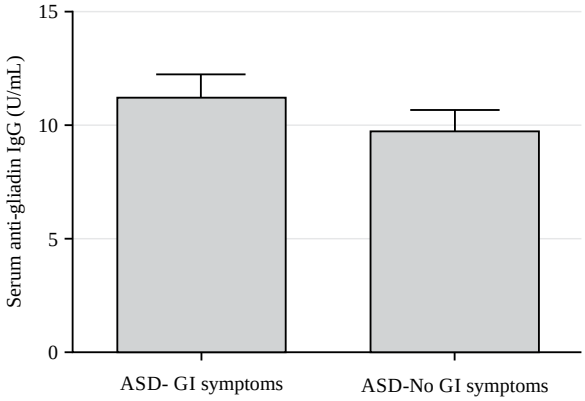

Fig. 3: Anti-gliadin IgG levels in relation to GI complaints

No significant difference in IgG levels between ASD patients who complain of GI symptoms and ASD patients without any GI complaints and p = 0.30

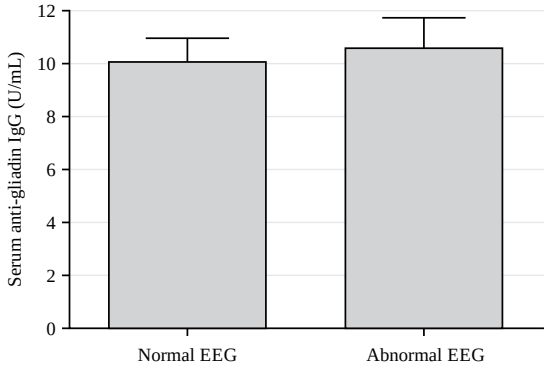

Fig. 4: Anti-gliadin IgG levels in relation to EEG abnormalities

Figure shows no significant difference in IgG levels between ASD patients who have normal and abnormal EEG records and p = 0.69

associated with ASD was also tested, i.e., GI complaints and EEG abnormalities using Pearson's correlation coefficient. No significant correlation was found between anti-gliadin IgG levels and the presence of GI complaints nor EEG abnormalities in children with ASD (r = -0.026 and r = -0.023, respectively, p>0.05) (Fig. 3-4).

Table 1: Study sample descriptive statistics

Group	Number of participants (N)		Mean age±SEM
ASD	Total	319	6.2±0.12
	Female	74	5.7±0.18
	Male	245	6.4±0.15
Siblings	Total	172	6.0±0.15
	Female	51	5.6±0.24
	Male	121	6.1±0.19
UHC	Total	327	6.3±0.14
	Female	97	6.3±0.22
	Male	230	6.3±0.17

ASD: Autistic spectrum disorders and UHC: Unrelated healthy controls

DISCUSSION

The current study revealed no significant difference in IgA and anti-TTG IgA levels among the three groups, suggesting that the prevalence of celiac disease in patients diagnosed with ASD doesn't differ significantly from the prevalence of celiac disease in the normal population. This finding has been supported by previous studies that have similar results in which Pavone *et al.*[17] suggested that the occurrence of ASD and celiac disease is a pure coincidence. Nevertheless, opposing findings in the literature were found[18]. The current study data is indicative that performing routine screening for celiac disease in patients with autism is neither cost-effective nor justifiable.

Another finding was the increased levels of IgG antibodies to gliadin in two groups; patients with autism and their siblings. This increase was statistically significant. However, there was no association with the control group. This suggests that high levels of IgG do not necessarily mean gluten sensitivity. Similar results were also reported by Jácome *et al.*[19]. The fact that the increase in IgG was proved in the healthy siblings' group, as well as the ASD group, may imply that the increase could be attributed to genetics. One possibility is that both patients and their siblings usually would have been exposed to the same food and this resulted at some point in the past to a triggered IgG antibody by gliadin. However, this is not dependent on continuous exposure to gliadin as IgA levels are not significantly increased. In different studies, the exact percentage of children with ASD who complain of gastrointestinal symptoms varies, but these studies agree that gastrointestinal abnormalities are common in ASD[20]. Marí-Bauset *et al.*[21] reported that the immature gut mucosal immune system in children makes them more sensitive to certain food proteins, this might explain the high incidence of gastrointestinal abnormalities. In this study, the association between gastrointestinal symptoms and IgG concentration is not significant, this will enforce the findings of many previous studies. Autoimmune activity in patients with ASD was found to be related to their prefrontal cortex maldevelopment, critically in the postnatal development period. This can induce abnormal neural connectivity that is measured by electroencephalography (EEG)[22]. In the current study, the measured EEG to IgG levels in those patients and found no significant relationship. This study provides many strong points including the new ethnicity studies have never been studied before, a large sample of subjects with different age groups, including the sibling group as a control group to decrease confounding factors such as exposure to a similar variety of foods, socio-economic factors, as well as hereditary factors. However, this study has some limitations including that the gut permeability was not tested in these patients to correlate with IgG levels and no pathological samples were tested from the intestine to correlate with IgG levels or symptoms.

CONCLUSION

Most of the tested markers of gluten sensitivity showed no association with ASD. Furthermore, anti-gliadin IgG levels show higher levels in ASD compared to UHC. It was also elevated in the sibling group with no statistical difference compared to ASD, undermining its role in the pathophysiology of ASD. Also, the elevated levels of IgG have not been correlated with any ASD-associated symptoms such as GI complaints or EEG abnormalities. All the above findings suggest that this elevation could be due to lifestyle, food intake, or genetic factors rather than ASD. Therefore, routine screening for gluten sensitivity markers in patients with ASD is neither cost-effective nor justifiable.

SIGNIFICANCE STATEMENT

Autism Spectrum Disorder (ASD) is a group of neurodevelopmental disabilities that lack a clear etiology. To date, studies investigating the role of immune reactivity to gluten in ASD have been inconsistent. The current study sought to examine gluten-related immune reactivity in a sample of children diagnosed with ASD without gastrointestinal symptoms. In the current study the anti-gliadin IgG levels were significantly higher in ASD patients and their siblings compared to the healthy group. Also, those IgG levels were not associated with gastrointestinal complaints or electroencephalogram abnormalities.

ACKNOWLEDGMENTS

The Deanship of Academic Research supported this research at the University of Jordan, Amman, Jordan (10/2018/1181). We thank Ms. Suzan Albdour for her technical assistance. The second author passed away on Feb. 7th of this year and was an essential part of this project. This manuscript is dedicated to his soul.

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