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Measures of gluten-related reactivity in children with autism spectrum disorders in the absence of overt gastrointestinal symptoms: a pilot study from the United Arab Emirates

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Abstract

Objectives: The aetiology of autism spectrum disorder (ASD) is multifactorial, sometimes genetic, and may be associated with abnormal immunological responses to peptides from

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proteins such as gluten. These peptides may cross the blood-brain barrier and affect neurotransmission, resulting in behavioural symptoms consistent with ASD. The aim of this study was to screen for markers of gluten-related immune reactivity in the absence of overt gastrointestinal symptoms in patients with ASD in the United Arab Emirates, a country associated with a high prevalence of ASD but lacking this type of research.

Methods: Patients diagnosed with ASD (using Diagnostic and Statistical Manual of Mental Disorders-IV-based criteria and Autism Diagnostic Observational Schedules) were compared with controls, regarding anti-tissue transglutaminase (tTG) immunoglobulin (Ig) A and anti-deamidated gliadin peptide (DGP) IgA levels.

Results: Sixty-six patients with ASD and 101 controls were included. Patients with ASD showed statistically significant lower anti-DGP IgA levels, but no significant difference in anti-tTG IgA levels, versus healthy controls. Correlations between immunological data and clinical symptoms were synergistic, but not statistically significant.

Conclusion: ASD may be associated with reduced levels of anti-DGP IgA.

Keywords

Autism spectrum disorders, opioid excess theory, gluten, immunoglobulins, neurotransmission, anti-tissue transglutaminase antibody, anti-gliadin antibody

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Introduction

Autism spectrum disorder (ASD) is a group of conditions with an estimated prevalence up to around 1.5% in developed countries.[1–3] In children under 5 years of age, ASD is considered the leading cause of disability among all mental disorders, and the fourth in children aged 5–14 years.[1] The United Arab Emirates has been reported to have the highest estimated disability-adjusted-life-years rates for ASD compared with Western Europe (137/100 000 versus 99/100 000).[1] One of the challenges in ASD research is to identify plausible aetiological causes, particularly in view of the phenotypical heterogeneity of the disorder and the high rates of comorbidity.[4]

One theory postulates the contribution of the gut-brain axis to ASD, based on the observation that patients with ASD often experience a range of gastrointestinal disorders, including coeliac disease, food allergies, and other malabsorptions.[5,6] A proportion of biologically active peptides might cross the blood-brain barrier and interfere with the gut-brain axis.[6,7] In 1979, Panksepp suggested that peptides mimicking endogenous opiates may explain some ASD symptoms, such as decreased pain sensitivity, reduced desire for social contact and repetitive behaviours (e.g. self-injurious behaviour),[8] which are ameliorated by naltrexone in a subgroup of patients.[9–11] More recently, gluten proteins originating from wheat have been implicated as main agents, passing through the intestine and the blood-brain barrier into the brain. Elevated levels of gluten exorphins have been found in the urine samples of patients with autism,[12] and may be an expression of increased gut permeability to these peptides. The theory implies a state of gluten immune response,

defined as an enhanced immunologic reaction to gluten proteins.[13] A 5-fold increased risk of altered serological tests specific for gluten related disorders, such as coeliac disease, has been reported in the early diagnosis of autism, even in the absence of inflammatory changes in the small intestine.[14] Measurement of anti-tissue transglutaminase (tTG) and anti-deamidated gliadin peptide (DGP) immunoglobulins can provide a first line serological approach to identify coeliac disease,[15] and individuals affected by non-coeliac gluten sensitivity may also test positive to raised levels of these immunoglobulins.[16] To date, some studies have shown elevated anti-tTG and anti-DGP immunoglobulins in children with autistic disorders, while others have not.[17–20]

The aim of the present study was to investigate gluten-related immune reactivity in a group of children diagnosed with ASD in the absence of gastrointestinal symptoms, in a part of the world where this type of research has not yet been conducted. This is of interest because epidemiological differences in ASD may also translate to gut-brain-axis activity.[21,22] Levels of anti-tTG and anti-DGP immunoglobulin (Ig) A were measured in children with ASD and compared with healthy children. To the best of the authors' knowledge, this is the first study from the United Arab Emirates to investigate levels of markers of gluten-related immune reactivity in children with ASD. Higher levels of anti-tTG and anti-DGP IgAs in children with ASD, in the absence of gastrointestinal symptoms, was predicted to be suggestive of gluten sensitivity, and supportive of gut-brain-axis abnormalities in the aetiology of ASD.

Patients and methods

Study population and procedures

This case–control study was conducted at Al Ain Hospital, a major regional hospital in the Abu Dhabi region linked with the United Arab Emirates University (UAEU), College of Medicine and Health Sciences, Department of Psychiatry. The study followed 'Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)' guidelines (https: //www.equator-network.org/reporting-guidelines/strobe/). All male and female patients aged $\leq$16 years, referred to the Child Psychiatry Clinic at Al-Ain Hospital (Emirate of Abu Dhabi, UAE) with a confirmed diagnosis of ASD, were consecutively recruited between January 2013 and January 2016. Control subjects were recruited from randomly selected healthy siblings of children visiting the outpatient clinic for conditions other than ASD. To confirm diagnosis, children with suspected ASD were administered the Autism Diagnostic Observational Schedules (ADOS), a semi-structured tool for assessing individuals with suspected autism or other pervasive developmental disorders.[23] Patients were included if they met the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria for ASD, and if they were free from any co-morbid DSM-IV Axis I condition and did not experience any gastrointestinal tract disorder according to their medical records. Participants were excluded from the study if they were aged >16 years, and/or if they were diagnosed with any physical conditions, or received any treatment that may affect the response of the immune system, or if they were receiving any special diet. Ethics approval for the study was obtained from the institutional review board at Al Ain Hospital, Al Ain City, Abu Dhabi, United Arab Emirates, in accordance with the Declaration of Helsinki (2000) (REC number: AAHEC-12-15-034, 27/01/2016). Written informed consent was obtained from the parents of all study participants.

All participants underwent a clinical assessment, conducted between 08.00 h and 11.00 h. Venous blood samples (5 ml) were

collected and allowed to clot at room temperature for 30 min for serum preparation. Immediately following clinical assessment, serum samples were assessed for IgAs against tTG and DGP using relevant commercial enzyme-linked immunosorbent assays (Orgentec Diagnostica, Mainz, Germany) as per the manufacturer's instructions. Absorbances of resultant colour reactions were determined at 450 nm (with a reference reading at 620 nm), and converted to IgA values in U/ml. Values greater than 15 U/ml (tTG) and 12 U/ml (DGP) were considered positive, as established by the manufacturer.

Statistical Analyses

Data are presented as mean ± SD, and were analysed using IBM SPSS software for Windows, version 25.0 (IBM, Armonk, NY, USA). Between-group differences in demographic and clinical data were analysed using χ^2-test and independent-samples *t*-test. Biochemical and clinical data were assessed for normality and Log transformed as necessary. Multivariate analysis of covariance within the General Linear Model in SPSS was used to compare case versus control serum antibodies, and to account for confounding variables. Pairwise differences (post-hoc analyses) were Bonferroni corrected for multiple comparisons. Relationships between ADOS scores and serum antibodies were explored using Pearson's correlation coefficient. Statistical significance was set to ensure 95% confidence intervals, conferring 0.80 power and an alpha error of 0.05 (two-tailed P value ≤ 0.05).

Results

Demographic and clinical characteristics

A total of 167 participants were included in the study, comprising 66 paediatric patients with ASD, and 101 healthy controls. The sample of patients with ASD represented 100% of the children diagnosed with ASD for the duration of the study. Demographic and clinical variables are presented in Table 1. Age and sex differed between the two groups and were accounted for as confounders in the general linear model analyses.

Anti-tissue transglutaminase IgA and anti-deamidated gliadin peptide IgA

Assays of serum IgA levels (U/ml) showed higher mean levels of anti-tTG and lower mean levels of anti-DGP in patients with ASD versus healthy controls (Table 1 and Figure 1). Multivariate analyses, after controlling for age and sex, indicated a

Table 1. Demographic and clinical characteristics of paediatric patients with autism spectrum disorder (ASD) and healthy controls (HC).

Characteristic	ASD $n = 66$	HC $n = 101$	Statistical significance
Age, years	3.8 ± 1.3	4.1 ± 1.6	$P < 0.001$
Sex, male/female	53/13	60/41	$P < 0.005$
UAE native/non-native	35/31	60/41	$P = 0.4$
Anti-tTG IgA, U/ml	1.22 ± 2.15	0.95 ± 0.88	$P = 0.32$[a]
Anti-DGP IgA, U/ml	2.79 ± 3.1	3.85 ± 4.43	$P = 0.006$[a]

Data presented as mean ± SD or *n* prevalence.

UAE, United Arab Emirates; IgA, immunoglobulin A; tTG, tissue transglutaminase; DGP, deamidated gliadin peptide.

[a]Between-group effects with Bonferroni correction following multivariate analyses ($P = 0.011$) controlling for age and sex.

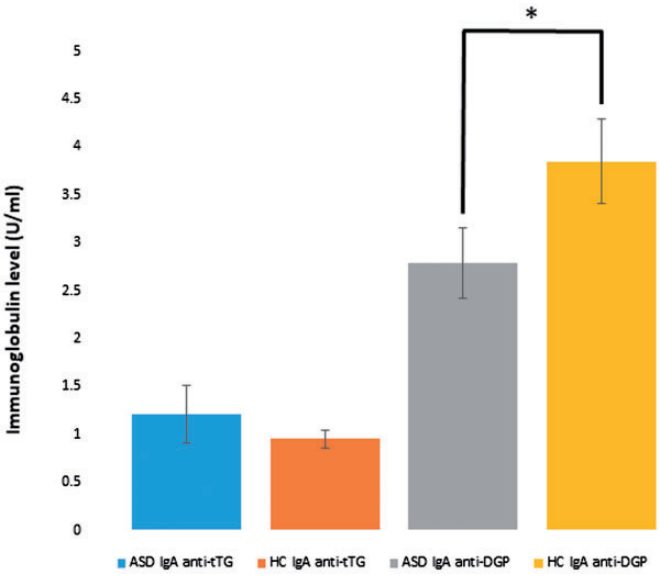

Figure 1. Levels of serum anti-tissue transglutaminase (tTG) immunoglobulin (Ig)A and anti-deamidated gliadin peptide (DGP) IgA in patients aged $\leq$ 16 years with autism spectrum disorder (ASD) and healthy controls (HC). Data presented as mean $\pm$ SE; *statistically significant difference ($P = 0.006$).

statistically significant difference at group level ($F = [2, 162] = 4.68$, $P = 0.011$). Between-group analyses with Bonferroni correction indicated that this difference was driven by anti-DGP IgA levels reaching statistical significance ($P = 0.006$), whilst differences in anti-tTG IgA levels did not reach statistical significance ($P = 0.32$). No participant from either group showed clinically abnormal anti-tTG IgA levels (>15 U/ml). Two patients with ASD (3.0%) and five heathy control participants (5.0%) displayed anti-DGP IgA levels above the cut-off value (>12 U/ml).

Correlation analyses

No statistically significant correlation was found between ADOS scores and levels of anti-DGP IgA ($r = -0.065$, $P = 0.62$) or levels of anti-tTG IgA ($r = 0.014$, $P = 0.91$). In addition, there was no statistically significant correlation between levels of anti-DGP and anti-tTG IgA ($r = 0.093$, $P = 0.23$).

Discussion

The aim of the present study was to screen for serological markers of gluten-related immune reactivity in a group of children with ASD in the absence of overt gastrointestinal symptoms compared with healthy individuals. To the best of the authors' knowledge, this is the first such investigation carried out in the United Arab Emirates. The analyses indicated statistically significant lower anti-DGP IgA in children with ASD versus healthy children. Differences in anti-tTG IgA levels were not statistically significant between the two groups. In addition, there were no statistically significant correlations between ant-tTG and anti-DGP IgA levels, or between IgA levels and ADOS scores, although the direction of signal was inverse for anti-DGP

IgA and positive for anti-tTG IgA in relation to ADOS scores.

Published evidence supports the role of the brain-gut axis in ASD, based on the frequent occurrence of gastrointestinal symptoms in children with autism (up to 70%) compared with children on a typical developmental trajectory (28%).[24–26] A number of case reports from the late sixties onwards introduced the possibility that a heightened immune response to gluten might contribute to ASD presentations.[27–32] In the general population, coeliac disease has a prevalence of approximately 1%,[15] and is associated with genes coding for human leukocyte antigens (HLA) DQ2 and DQ8,[33,34] and immune responses to deamidated epitopes of gliadin and tTG.[35] In the presence of gastrointestinal symptoms and in the absence of criteria for coeliac disease or evidence of allergy, the condition is termed nonceliac gluten sensitivity,[36] which lacks objective diagnostic tests.[36–38] People with nonceliac gluten sensitivity can, however, test positive for anti-DGP IgG.[16] The prevalence of nonceliac gluten sensitivity in the general population is known to range between 0.5% and 13%, which on average, is around 6 times higher than coeliac disease.[39]

Findings from the present study do not support immunological hyperactivity in children with ASD compared with healthy children. Although various studies report positive results, some investigations have not found a significant association between ASD and elevated IgA levels. For example, Pavone et al (1997),[19] found no elevated anti-gliadin levels in 11 patients with ASD. Out of 147 patients with ASD reported in another study, only one (0.68%) had abnormal anti-transglutaminase IgA levels and five (3.4%) had abnormal anti-gliadin IgA levels.[17] Furthermore, anti-transglutaminase IgA levels in a sample of 162 children with ASD and 44 healthy controls were not found to reach the threshold for clinical abnormality in either group.[18] The study also demonstrated that the prevalence of abnormal anti-DGP IgA was similar between children with ASD and healthy children, with only three children with ASD (2.3%) having clinically abnormal anti-DGP IgA.[18] Finally, Lau et al (2013),[13] found no significant difference in anti-DGP IgA in patients with ASD and their unaffected siblings versus healthy controls.

There are also inconsistencies in the literature investigating the relationship between coeliac disease and autism.[40] For example, one study demonstrated that the prevalence of coeliac disease in patients with autism was similar to 2 034 local healthy children.[17] Among the 147 patients with ASD, only six tested positive for anti-DGP IgA or transglutaminase antibodies, whilst all participants tested negative for endomysium antibodies. Additionally, in this sample, the prevalence of ASD was not significantly greater than the general population when coeliac disease was confirmed by biopsy.[17] Another study demonstrated no strict correlation between coeliac serology and ultrastructural changes in pathology.[19] Out of 120 patients with coeliac disease, the prevalence of ASD was not greater than expected in patients with coeliac disease, whereas patients positive for anti-DGP and endomysium antibodies had normal intestinal mucosa.[19] Interestingly, a very large epidemiological study from Sweden established that, although there was no association between coeliac disease or inflammation and ASD, the risk of ASD was significantly higher in the case of normal mucosa in combination with a positive coeliac disease serology.[14]

Although the cross-sectional nature of the present work precludes any comment regarding the nature of the association between ASD and anti-DGP IgA, the

study does not support nonceliac gluten sensitivity in the present sample of children with ASD, based on the serology utilized. It is possible that the slightly lower anti-DGP IgA measured in the present study may be driven by a gluten-free diet,[41] as diet was not evaluated. However, the role of gluten and a gluten-free diet in individuals with ASD remains unclear,[42] and some studies evaluating diets have methodological failings.[43] Nevertheless, children on these diets may potentially have less immune reactivity to gluten-containing food products, which might result in underrepresentation of patients with elevated gluten-related immunological markers, or favour lower than expected measurements.[44] Salivary IgA has been proposed as an alternative biomarker for research on the mucosal immune system, as it appears independent from diet, although the authors of this proposal did not specifically test for anti-DGP or anti-tTG IgAs.[45] It is possible that there may be subgroups of patients with ASD who have different phenotypical manifestations of immune system dysregulation. Children with ASD and enhanced immunological activation may be those with either overt gastrointestinal symptoms (excluded from the present study) or those not yet symptomatic but at risk of developing coeliac disease or nonceliac gluten sensitivity. In view of the frequent occurrence of gastrointestinal symptoms in children with ASD, it might be valuable to screen their HLA status as early as possible to help identify individuals who are non-symptomatic at the time of ASD diagnosis (as in the present clinical sample) but who are at risk of developing symptoms later in life. Other explanations for the immune system being involved in the aetiology of ASD include the possibility that immunological dysfunction may be genetically driven, resulting in antibodies against self (e.g. central nervous system proteins), may follow maternal immune activation, or may be the result of potentially inefficient immunological responses to external pathogens.[46,47]

The results of the present study may be limited by several factors, including the lack of HLA genotyping of participants, and lack of dietary intake control, which might have influenced the results. Furthermore, as the analyses did not correct for potential differences in total IgA levels, and secretory IgAs were not measured, it is not possible to comment on this parameter. It would have been preferable to match the present sample for sex and age at baseline, and to have access to detailed information regarding ethnicity, however, the analyses controlled for age and sex, and an overly conservative approach was adopted, utilizing Bonferroni correction when setting the threshold for statistical significance. A power calculation could not be performed in the absence of clear epidemiological data on the occurrence of nonceliac gluten sensitivity in children with ASD. The present data do allow a power calculation to be performed in further studies, which should be useful to generate new hypotheses. Strengths of the present study include the relatively large study population, and the inclusion of a medication free, homogenous group of individuals with no psychiatric or physical comorbidity.

In conclusion, this is the first study from the United Arab Emirates to compare anti-tTG and anti-DGP IgAs in paediatric patients with ASD versus healthy controls. Statistically significantly lower anti-DGP IgA levels were shown in patients with ASD. Future studies investigating the coeliac status of individuals with ASD with or without gastrointestinal symptoms, using a preferentially longitudinal approach, might help clarify the relationship between the gut-brain axis and ASD in relation to gluten.

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Declaration of conflict of interest

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