Gluten sensitivity in autistic children in Central Saudi Arabia

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ABSTRACT

Objectives: To examine gluten sensitivity through the measurement of anti-gliadin antibody (IgA and IgG), anti-endomysial antibody (endo-IgA), anti-reticulin antibody (IgG) and anti-transglutamase antibody (IgG) levels in blood samples of autistic in the Riyadh area.

Methods: The study took place in the Department of Physiology, Faculty of Medicine, King Saud University, Riyadh between September 2003 and April 2004. Thirty-three autistic children, from the Riyadh area participated in the study, all with confirmed diagnosis according to E-2 diagnostic criteria for autistic spectrum disorders. Anti-gliadin antibody (IgA and IgG), anti-endomysial antibody (endo-IgA), anti-reticulin antibody (IgG) and anti-transglutamase antibody (IgG), were measured by the enzyme linked immunosorbent assay method.

Results: Thirty-three autistic children with confirmed diagnosis (30 males and 3 females) participated in the study. A significant percentage of autistic children complained of constipation as compared to control. None of the autistic examined were positive for any of the antibodies tested, including anti-gliadin antibody (IgA and IgG), anti-endomysial antibody (endo-IgA), anti-reticulin antibody (IgG) and anti-transglutamase antibody (IgG). The same results were reached with the control group.

Conclusions: The present study demonstrated that gluten sensitivity is not a major cause in those autistic children examined.

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Autism is a neuro-developmental behavioral disorder with onset before 36 months, characterized by impairment of social contact and communication, restricted and repetitive interest and behaviors.[1] Other characteristics include, sensory dysfunction, inappropriate laughing and giggling, little or no eye contact, apparent insensitivity to pain, preference to be alone and many more, according to the American Psychiatrist Association. Recent epidemiological studies suggested that autism might affect one in 150 of children in the United States of America.[1,2] In the last 20 years, there has been an increase in the incidence of autism, unexplained by genetics alone, nor can this increase be secondary to only increased awareness. The etiology of autism is complex, and usually, the underlying pathologic mechanisms are unknown. Some scientists emphasize the possible impact of a number of postnatal factors ranging from environmental toxins to dietary factors.[2-5] We now recognize the gut-brain connection as a basic theory in autism. This theory suggests that peptides formed through the incomplete breakdown of foods containing gluten and casein (derived from dairy produce), exhibit direct opioid activity or form ligands for the peptidase enzymes, which break down endogenous endorphins and enkephalins. Individuals who cannot metabolize gluten produce A-gliadin, which they cannot metabolize further. This A-gliadin binds to A and D opioid receptors. We know now that those receptors associate with mood and

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behavior disturbances. Claims suggest a strict gluten free diet reduces the level of opioid peptides, and improves autism in some autistics.[6-8] Autistic children frequently develop gastrointestinal symptoms such as constipation, diarrhea, abdominal discomfort and distension.[9] Horvath et al[10] reported a 69% prevalence of histological esophagitis and a 58% prevalence of intestinal disaccharides deficiency in a group of 36 autistic children studied by upper gastrointestinal endoscopy and biopsy. Gluten sensitivity is an immune disorder triggered by environmental agents (gluten) in genetically predisposed people. Most patients who develop gluten sensitivity have a genetic predisposition for it. It associates with specific alleles at the HLA-DQ2 locus, found in 95% of sufferers.[11] Gluten in food results in a T-cell-mediated inflammatory response in the proximal small bowel that damages the mucosa and leads to malabsorption. The inflammatory response continues as long as patients continue to ingest protein.[12] Recently, a tissue enzyme transglutaminase was identified as the autoantigen of gluten sensitivity. Interestingly, gliadin is a preferred substrate for this particular enzyme, giving rise to novel antigenic epitopes. Immunological detection of IgA and IgG antibodies to tissue transglutaminase (specific peptide), the newly discovered autoantigen to gluten sensitivity, is a useful tool in the diagnosis and follow-up of the disease.[13] Since autism is one of the disorders that causes impairment in children of Saudi Arabia and its etiology is still not clear, the aim of this study is to establish whether gluten sensitivity is a possible pathophysiological factor in the development of autism in our area. To reach this goal, we will assess the serological response to ingested gluten through the measurement of anti-gliadin antibody (IgA and IgG), anti-endomysial antibody (endo-IgA), anti-reticulin antibody (IgG) and anti-transglutamase antibody (IgG), exploring possible correlation between gluten sensitivity and autism.

Methods. The study was conducted in the Department of Physiology, Faculty of Medicine, King Saud University, Riyadh between September 2003 and April 2004. Thirty-three autistic children, aged up to 12 years, with confirmed professional diagnoses were selected. The diagnosis was carried out either by a qualified psychologist, psychiatrist or neurologist, according to diagnostic criteria E-2.[14] Written consent was obtained from their parents prior the start of the study, and then the parents were asked to complete a questionnaire regarding the child's medical and behavioral history. Healthy age and sex matched control volunteers were recruited from King Abdul-Aziz University Hospital. Blood samples were collected by venipuncture in plain tubes, and after gentle mixing allowed to clot at room temperature, and then centrifuged at 1,500 g for 10 minutes. Then serum was collected and stored at –70°C until assay time. Samples were assayed in a single large batch, in duplicate. Anti-gliadin antibody (IgA and IgG), anti-endomysial antibody (endo-IgA), anti-reticulin antibody (IgG) was measured by a commercially available ELIZA kit (Diagnostic Systems, Texas, USA). Anti-transglutamase antibody (IgG) was measured by a commercially available ELIZA kit from (IBL Immunodiagnostics-Biological Laboratories, Hamburg). Results were analyzed using SPSS for windows and expressed as mean ± SEM. Statistical analyses for differences among the groups were assessed by t-test. *P*-values of ≤ 0.05 were considered significant.

Results. Thirty-three autistic children with confirmed diagnosis (30 males and 3 females) participated in the study. The mean age for the total number of autistic children was 7.4±0.5 years. The male to female ratio was 10 to 1. **Table 1** illustrates the percentages of gastrointestinal symptoms in autistics and control children. None of the autistics examined were positive for any of antibodies tested, including anti-gliadin antibody (IgA and IgG), anti-endomysial antibody (endo-IgA), anti-reticulin antibody (IgG) and anti-transglutamase antibody (IgG). The same results were reached with the control group.

Discussion. Results from our study demonstrated no significant differences in the incidence of abdominal pain, vomiting or chronic diarrhea, among autistic children compared with control children. However, a significantly higher number of autistics complained of constipation compared with control children. Furthermore, our results indicated that both autistic

Table 1 - Percentage of gastrointestinal symptoms in autistics and control children.

Gastrointestinal symptoms	No. (%)	
	Control	Autistic
Chronic abdominal pain	9 (27)	12 (36)
Chronic vomiting	6 (18)	9 (27)
Chronic diarrhea	9 (27)	12 (36)
Constipation	6 (18)	21 (64)*

*Significant

children and control children examined in this study have no gluten sensitivity, through the absence of anti-gliadin antibody (IgA and IgG), anti-endomysial antibody (endo-IgA), anti-reticulin antibody (IgG) and anti-transglutamase antibody (IgG). There are contradicting results reported regarding the connection between gluten sensitivity and autism. Sponheim in 1991,[15] reported no connection between gluten and behavior in autistic and children with hyperactivity. Actually, Sponheim thought that a gluten free diet could be another negative factor leading to further social isolation in this group of highly socially handicapped patients and families.[15] Moreover, Cunningham and Marcason,[16] concluded that there is insufficient evidence to support the beneficial effect of casein and gluten free diet in autistics. But, Reichelt et al,[9] Knivsberg et al,[17,18] Cummins et al,[19] and many others claimed that gluten sensitivity could play an important role in the pathophysiology of autism.

Gastrointestinal diseases are more common in children with a neurological disability,[20] and previous reports describe unexpected intestinal inflammation, with low-grade colitis,[21,22] and duodenitis with reduced disaccharides in children with autism. Immunohistochemical studies suggest an immune response targeted at the gut epithelium, with a possible autoimmune cause.[23,24] An early report from Melmed et al,[22] of a large unselected population of autistic children, however, identified approximately half with gastrointestinal problems when formal assessment was made.[22] Immunologic hypersensitivity to the gliadin component of wheat and other cereal grains causes gluten-sensitive enteropathy. This results in malabsorption and fatty diarrhea. The consequences of malabsorption are weight loss, nutritional deficiencies, and growth failure in children.[25] The gastrointestinal (GI) lesion responds rapidly to gluten exclusion from the diet, both clinically and histologically.[26] The inflammatory lesion or villous atrophy induced by gliadin peptide resulted in activation of t-cells in Peyer's patches via specific HLA-DQ restriction molecules. These activated t-cells migrate to lamina propria where they secrete cytokines, such as TNF-α, upon restimulation and cause damage to the tissue. The communication of activated t-cells with b-cells, results in the production of IgA antigliadin antibodies. High tissue titers of IgA antigliadin antibodies are quite specific for gluten-sensitive enteropathy and occur in the vast majority of patients.[27] Constipation is a relatively common problem in children, with estimates of prevalence in otherwise, normal children reaching up to 8% and 30% in autistic children.[28] The significantly higher incidence of constipation observed in autistic children examined in this study, could be due to the fact that they are very meticulous eaters with eating problems, most of them refusing to eat fruits and vegetables. Some scientists reported an improvement in social interaction, communication and imaginative skills in autistic children on gluten free diet as compared to control.[29] However, Pavone et al,[30] failed to find any connection between autism and sensitivity to gluten. In a study conducted on 8 autistic patients with steatorrhea, hypocalciuria, and alleged behavioral improvements on gluten restriction, they were fed ordinary diets plus 20 g gluten/day for 4 weeks. None of the patients had any significant change in body-weight or bowel habit as a result of gluten challenge, nor were any histological abnormalities detected on jejunal biopsy. They concluded that the steatorrhea and hypocalciuria seen in some autistic subjects could not be accounted for by the presence of gluten sensitivity. The researchers insisted that these patients should not be confined to gluten-free diets, unless rigorous behavioral studies demonstrate a statistically significant improvement in behavior because of the diet, or deterioration during challenge.[31]

Gluten sensitivity principally affects people of European heritage, with an estimated prevalence of 1/300 in the United Kingdom and similar prevalence reported in Italy, Sweden, and Australia.[11] The highest incidence reported is in Northern Ireland at 1/150.[32] Gluten sensitivity is rare in people of other ethnic backgrounds (including Arabs) and exceedingly rare in pure Japanese, Chinese and Afro-Caribbean descent.[33] This might explain the negative results which we reached in the current study. Furthermore, it might indicate an important fact that although causes of autism are the same world wide, its ranking order is distinct in different parts depending on the environmental and genetic factors. As a result, gluten sensitivity might be not a major factor in the pathophysiology of autism in the Riyadh area. One of the main limitations of the study is the small sample size, and further work on a large scale is recommended.

References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington DC; American Psychiatric Association: 1994.
2. Wing L. The autistic spectrum. ***Lancet*** 1997; 350: 1761-1766.
3. Trottier G, Srivastava L, Walkev CD. Etiology of infantile autism. A review of recent advances in genetic and neurological research. ***J Psychiatry Neurosci*** 1999; 24: 103-115.
4. Fein D, Joy S, Green L, Waterhouse L. Autism and pervasive developmental disorders. In: Fogel B, Schiffer R, Rao S, editors. Neuropsychiatry. Baltimore (MD): Williams & Wilkins; 1996.

5. Waterhouse L, Fein D, Modahl C. Neurofunctional mechanisms in autism. ***Psychol Rev*** 1996; 103: 457-489.
6. Binstock T. Anterior insular cortex: linking intestinal pathology and brain function in autism-spectrum subgroups. ***Med Hypotheses*** 2001; 57: 714-717.
7. Shattock P, Lowdon G. Proteins, Peptides and Autism. Part 2: Implications for the Education and Care of People with Autism. ***Brain Dysfunction*** 1991; 4: 323-334.
8. Wakefield AJ, Puleston JM, Montgomery SM, Anthony A, O'Leary JJ, Murch SH. The concept of entero-colonic encephalopathy, autism and opioid receptor ligands. ***Aliment Pharmacol Ther*** 2002; 16: 663-674.
9. Reichelt KL, Scott H, Ekrem J. Gluten, milk proteins and autism: the results of dietary intervention on behavior and peptide secretion. ***J Appl Nutr*** 1990; 42: 1-11.
10. Horvath K, Papadimitriou JC, Rabsztyn A, Drachenberg C, Tildon JT. Gastrointestinal abnormalities in children with autistic disorder. ***J Pediatr*** 1999; 135: 559-563.
11. Logan RF. Descriptive epidemiology of celiac disease. In: Branski D, Rozen P, Kagnoff MF, editors. Gluten sensitive enteropathy. Basel: Karger; 1992: 1-14.
12. Bernstein CN, Leslie WD, Leboff MS. AGA technical review on osteoporosis in gastrointestinal disease. ***Gastroenterology*** 2003; 124: 795-841.
13. Sulkanen S, Hattunen T, Laurila K, Kolho KL, Korponay-Szabo IR, Sarnesto A et al. Tissues transglutamase as autoantibody enzymes-linked immunosorbent assay in detecting celiac disease. ***Gastroenterology*** 1998; 115: 1322-1328.
14. Rimland B. On the objective diagnosis of infantile autism. ***Acta Paedopsychiatr*** 1968; 35: 146-161.
15. Sponheim E. Gluten-free diet in infantile autism. A therapeutic trial. ***Tidsskr Nor Laegeforen*** 1991; 111: 704-707.
16. Cunningham E, Marcason W. Question of the month: Is there any research to support a gluten and casein free diet for child that is diagnosed with autism. American Dietetic Association. ***J Am Diet Assoc*** 2001; 2: 222-227.
17. Knivsberg AM, Miig K, Lind G, Nodland M, Reichelt K. Dietary intervention in autistic syndromes. ***Brain Dysfunction*** 1990; 3: 315-317.
18. Knivsberg AM, Reichelt K, Nodland M, Hoien T. Autistic Syndromes and Diet: A Follow-Up Study. ***Scandinavian Journal of Educational Research*** 1995; 39: 223-236.
19. Cummins A, Pentilla IA, Labrooy JT, Robb TA, Davidson GP. Recovery of the Small Intestine in Celiac Disease on a Gluten-Free Diet: Changes in Intestinal Permeability, Small Bowel Morphology and T-Cell Activity. ***J Gastroenterol Hepatol*** 1991; 6: 53-57.
20. Sullivan PB. Gastrointestinal problems in the neurologically impaired child. ***Baillieres Clin Gastroenterol*** 1997; 11: 529-546.
21. Torrente F, Ashwood P, Day R, Machado N, Furlano RI, Anthony A et al. Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. ***Mol Psychiatry*** 2002; 7: 375-382.
22. Melmed RD, Schneider CK, Fabes RA, Philips J, Reichelt K. Metabolic markers and gastrointestinal symptoms in children with autism and related disorders [abstract]. ***J Pediatr Gastroenterol Nutr*** 2000; 31 (Suppl 2): S31-S32.
23. Dalrymple NJ, Ruble LA. Toilet training and behaviors of people with autism: parent views. ***J Autism Dev Disord*** 1992; 22: 265-275.
24. Murch S, Thomson M, Walker-Smith J. Autism, inflammatory bowel disease, and MMR vaccine. ***Lancet*** 1998; 351: 908-912.
25. Papadopoulou A, Clayden GS, Booth IW. The clinical value of solid marker transit studies in childhood constipation and soiling. ***Eur J Pediatr*** 1994; 153: 560-564.
26. Lucarelli S, Frediani T, Zingoni AM. Food allergy and infantile autism. ***Panminerva Med*** 1995; 37: 137-141.
27. Knivsber AM, Reichelt KL, Nodland M. Reports on dietary intervention in autistic disorders. ***Nutr Neurosci*** 2001; 4: 25-37.
28. Baker SS, Liptak GS, Colletti RB. Constipation in infants and children: evaluation and treatment. A medical position statement of the North American Society for Pediatric Gastroenterology and Nutrition. ***J Pediatr Gastroenterol Nutr*** 1999; 29: 612-626. Published erratum appears in ***J Pediatr Gastroenterol Nutr*** 2000; 30: 109.
29. Knivsberg AM, Reichelt KL, Hoien T, Nodland M. A randomized, controlled study of dietary intervention in autistic syndromes. ***Nutr Neurosci*** 2002; 5: 251-261.
30. Pavone L, Fiumara A, Bottaro G, Mazzone D, Coleman M. Autism and celiac disease: failure to validate the hypothesis that a link might exist. ***Biol Psychiatry*** 1997; 42: 72-75.
31. McCarthy DM, Coleman M. Response of intestinal mucosa to gluten challenge in autistic subjects. ***Lancet*** 1979; 2: 877-878.
32. Ciclitira PJ, King AL, Fraser JS. AGA technical review on celiac sprue. American Gastroenterological Association. ***Gastroenterology*** 2001; 120: 1526-1540.
33. Devlin SM, Christopher NA, Paul LB. Celiac disease CME update for family physician. ***Can Fam Physician*** 2005; 50: 719-725.