Effect of gluten free diet on gastrointestinal and behavioral indices for children with autism spectrum disorders: a randomized clinical trial

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Background: **Genetic and environmental factors are both responsible for the etiology of autism spectrum disorders (ASD). Although epidemiological studies have been conducted to clarify the association between restriction diets and ASD, the conclusion remains unclear. This study was undertaken to investigate the effect of gluten free diet (GFD) on gastrointestinal symptoms and behavioral indices in children with ASD.**

Methods: **In this randomized clinical trial, 80 children diagnosed with ASD by the Autism Diagnostic Interview-Revised (ADI-R) were assigned into GFD (n=40) and regular diet (RD) (n=40) groups for 6 weeks. At the beginning and end of the intervention, the ROME III questionnaire for evaluating gastrointestinal symptoms and Gilliam Autism Rating Scale 2 questionnaire (GARS-2) for assessing psychometric properties were completed.**

Results: **Of the 80 children, 53.9% had gastrointestinal abnormalities. In the GFD group, the prevalence of gastrointestinal symptoms decreased significantly (P<0.05) after intake of GFD (40.57% *vs.* 17.10%) but increased insignificantly in the RD group (42.45% *vs.* 44.05%). GFD intervention resulted in a significant decrease in behavioral disorders (80.03±14.07 *vs.* 75.82±15.37, P<0.05) but an insignificant increase in the RD group (79.92±15.49 *vs.* 80.92±16.24).**

Conclusion: **This study suggested that GFD may be effective in controlling gastrointestinal symptoms and ASD behaviors.**

World J Pediatr June 2016; Online First

Key words: **autism;**
behavior disorders/problems;
gastrointestinal system;
gluten free diet

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doi: 10.1007/s12519-016-0040-z

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Original article

Introduction

Autistic spectrum disorders (ASD) (autistic disorder; pervasive developmental disorder, not otherwise specified; and asperger syndrome) are severe neurodevelopmental disorders with diagnostic features including qualitative impairment in social interactions (e.g., lack of social reciprocity, marked impairment in eye-to eye gaze, lack of joint attention), qualitative impairments in communication (e.g., lack of language development, echolalia, stereotyped, and repetitive use of language), and restricted repetitive and stereotyped patterns of behaviors, interests, and activities.[1]

According to the Autism and Developmental Disabilities Monitoring Network of Centers for Disease Control and Prevention, the incidence of ASD has increased from about 1/88 children in 2012 up to 1/ 68 children in 2014. This means that the estimated incidence of ASD increased by 30% during the period of 2012-2014. According to the the Centers for Disease Control, boys with ASD were identified five times as many as girls (1/54 *vs.* 1/252).[2] Iran is one of the countries with a low incidence of ASD and an incidence of 6.26/10000 in children of 5 years old.[3]

At present, the etiology of ASD is unknown; however, genetic and environment factors are implied.[4] ASD is a complex and clinically heterogeneous disorder with a spectrum of symptoms.[5] In the past, ASD was thought to be merely a psychological or neurological disorder. But at present, there is increasing evidence that it affects multiple systems of the body, for example the metabolic, gastrointestinal, immunological, mitochondrial, and

neurological systems.[6] Gastrointestinal co-morbidities are expressed as functional symptoms and chronic fundamental symptoms including celiac disease.[5] Researchers[7,8] believed that there is a link between behavioral disturbances and celiac disease in some cases of ASD. Celiac, also known as Gluten-sensitive enteropathy is a genetically linked autoimmune disorder in which eating certain types of grain-based products triggers an immune response that causes damage to the small intestine. Gluten is present in wheat, rye, barley, and malt.[9] Wheat is a major component of the Iranian diet (>150 kg per person per year). A high prevalence of celiac (1/166) has been found in Iran.[10]

As reported earlier, children with ASD often suffer from gastrointestinal discomforts such as esophageal reflux, abdominal pain, diarrhea, constipation, and bloating.[11] There have been few theories for gastrointestinal discomforts. The most important one is intestinal dysbiosis which is characterized by disruption of endogenous gut micro flora and overgrowth of pathogenic microorganisms. These microorganisms produce toxins that interact with neuron synapses.[12] Dysbiosis can also result from excessive antibiotic use.[6] Also, disruption in the mucosal lining of the gut and abnormal carbohydrate digestive enzyme activity lead to malabsorption of large proteins such as gluten, gliadin and casein, which may cause inflammation and are believed to act like neuropeptides and alter neurologic function.[12] Neuropeptides have adverse effects on attention, brain maturation, social interactions and learning.[13] These peptides bind to opioid receptors and, by modulating opioid levels in the brain, impair the central nervous system, a theory known as the "opioid peptide excess".[14-17] In children with ASD, peptides are not turned into amino acids and are presented in the urine, a variable known as urinary peptide level (UPL), which has been measured in many studies.[15] On the contrary, some experiments have not found abnormally high concentrations of opioid peptides in either plasma or the nervous system of patients with ASD.[18,19] Also, in few studies opioid peptides were not detected in the urine of children with autism.[20,21]

The similarities between the mechanisms involved in celiac disease and malabsorption of large proteins such as gluten, gliadin and casein proposed that there might be a link between celiac disease and ASD.[18] Goodwin et al[22] concluded that there was a correlation between autism and malabsorption and also between gluten sensitivity and cognitive impairment. Later on, McCarthy and Coleman[23] found no evidence for celiac disease in a small group previously considered to be gluten sensitive. This study found no association between autism and celiac disease.[23] In a case-control study, no association was found in 120 patients with celiac and non-celiac disease and in 11 patients with autism.[24] Patients on a gluten-free diet showed an improvement on a number of behavioral measures.[16,25] Antibodies to deamidated sequences of gliadin and autoantigen transglutaminase 2 are the two sensitive serological markers of celiac disease. Lau et al[26] found that the levels of celiac disease-specific serologic markers were not different between ASD patients and controls. However, there was increased immune activity to gluten. According to their study, the mechanism of abnormal immunological markers to gluten differed from that mentioned for celiac disease. The increased anti-gliadin antibody response and its association with gastrointestinal symptoms may be due to a mechanism involving immunologic and/or intestinal permeability. Research into restriction diets has excluded both gluten and casein because the breakdown products of these two molecules are similar.[21] In this study, gluten as a molecule that causes sensitivity in celiac and ASD patients was restricted. Our objective was partly to assess the effect of the gluten free diet. Therefore, the behavioral effect of casein has not been considered.

This study was to investigate the effect of the gluten free diet on gastrointestinal symptoms and behavioral indices in children with ASD.

Methods

Participants

This clinical trial included 80 children aged 4-16 years diagnosed psychiatrically with ASD. The diagnosis was confirmed by the autism diagnostic interview-revised (ADI-R) by a psychologist.[27] The children were recruited from the Iranian Special Education Organization for children with pervasive developmental disorders in Tabriz, Iran, after simple random sampling. Inclusion criteria were patients being diagnosed with ASD by a psychiatrist, aged 4-16 years, without a special restriction diet based on parent report. Exclusion criteria were patients with intellectual disabilities that were not diagnosed as ASD with ADI-R by a psychologist, children with feeding difficulties based on parent report, or inpatients and children with additional illnesses or abnormalities diagnosed by a psychiatrist.

These children were randomly divided into two groups with 40 children in each group, matched for age and sex. Diagnosis of gastrointestinal discomfort was based upon the Questionnaire on Pediatric Gastrointestinal Symptoms-Rome III version by a dietitian who was blinded to the study objective. Two of the children taking the gluten free diet (GFD) did not complete the study because their parents failed to keep them on the diet. Two children in the regular diet (RD)

group were also excluded. At last, there were 2 groups of 38 children. The first group received a GFD and the other group continued their RD for 6 weeks. The duration of the diet was based on the minimum period for achieving an acceptable result since it was the first time that a restricted diet was given to ASD patients in Iran.[21] The GFD consisted of gluten free pasta and biscuits and gluten free breads (Institute of Iranian Celiac Association, Registration Number 2895). The products were given weekly according to age requirement. Participants were categorized into 3 groups according to age: group 1 (4-8 years), group 2 (9-12 years), and group 3 (13-16 years). At the beginning of the week, the first group received 1 package of biscuits, 1 package of pasta and 4 loafs of bread. The second group received 2 packages of biscuits, 1 package of pasta, and 7 loafs of bread. The third group received 2 packages of biscuits, 1 package of pasta, and 9 loafs of bread. Also we called parents of the children frequently to ask if there were any problems regarding the diet. Additionally, a brochure containing a list of gluten free and gluten containing foodstuff and also recipes for preparing gluten free meals based on Iranian cuisine was presented to the parents.

At the beginning and end of the intervention, demographic information was gathered by interviewing children's parents. Gilliam Autism Rating Scale 2 (GARS-2) was also administered by a psychiatrist after interviewing children's parents. Furthermore, the ROME III questionnaire, the parent report version for children and adolescents, was completed. Written informed consent was obtained from parents. The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences and was registered in the Iranian Registry of Clinical Trials website (IRCT201404212017N20).

Measures

Autism Diagnostic Interview-Revised (ADI-R)

ADI-R which is administered in the form of a semi-structured interview with the child's primary caregivers covers early and current development features.[3] The ADI-R consists of 93 items arranged in three functional domains: language/communication; reciprocal social interactions; and restricted, repetitive, and stereotyped behaviors and interests.[27] The Persian version of this scale was standardized in 2005 with a reported Cronbach's alpha above 0.80.[28]

Gilliam Autism Rating Scale 2 (GARS-2)

GARS-2 is a behavior checklist designed for children and adolescents aged 3–22 years based on DSM-IV diagnostic criteria for Autism. The GARS-2 consists of 42 items (0-126 scores) grouped into three subscales: stereotyped behaviors, communication and social interaction. The Persian version was first reviewed for language clarity and appropriateness for use in Iranian culture with the cooperation of the Iranian Special Education Organization (ISEO). The tool was then pilot tested with 15 Iranian families from different socioeconomic backgrounds with a Chronbach alpha above 0.70.[29]

ROME III questionnaire (Parent Report Version for Children and Adolescents)

The clinical entities addressed in the ROME III questionnaire (0-191 scores) include (1) cyclic vomiting syndrome, rumination, and aerophagia; (2) abdominal pain-related disorders including functional dyspepsia, irritable bowel syndrome, abdominal migraine and functional abdominal pain; and (3) functional constipation and non-retentive fecal incontinence.[30] The ROME III questionnaire was translated into Persian and the accuracy of the translation was controlled by several courses in translation between Persian and English. The validity and reliability of the Persian questionnaire were tested in Damavand, Iran. The Cronbach alpha coefficient values were above 0.7 for all of the major symptoms included in the tool.[31]

Data analysis

The Statistical Package for Social Science (SPSS) version 16.00 was used for statistical analysis. The normality of variables was tested by the Kolmogorov–Smirnov test. Categorical variables were tested using the Chi-square test and continuous variables were tested using independent *t* test. Paired *t* test was used to compare the differences within a group or between the groups. Also covariate effects (age, gender) were adjusted by analysis of covariance (ANCOVA) using the general linear model. The statistical tests were two-sided, and a *P* value less than 0.05 were considered statistically significant.

Table 1. Demographic and health characteristics of study groups at baseline (*n*=76).

Variables	Total *n*=76	Gluten free diet *n*=38	Regular diet *n*=38	*P* value
Age (y), mean±SD	7.92±3.37	7.84±3.55	8.00±3.22	0.84
Age of diagnosis (y), mean±SD	3.90±2.25	3.55±2.02	4.25±2.44	0.18
Sex, *n* (%)				
Male	56 (73.7)	28 (73.7)	28 (73.7)	1.00
Female	20 (26.3)	10 (26.3)	10 (26.3)	1.00
Hyperactive, *n* (%)	37 (97.4)	16 (42.1)	21 (55.3)	0.25

SD: standard deviation. Categorical variables were analyzed by a two-sided Chi-square test and continuous variables by independent *t* test.

Results

Table 1 shows demographic and health characteristics. There was no statistically significant difference in any demographic variables including age, gender, age of diagnosis, and hyperactivity at the beginning of the intervention. The term hyperactivity was obtained from children's medical profile.

Gastrointestinal outcomes

According to the ROME III questionnaire, 53.9% of the patients had gastrointestinal abnormalities; 55.3% of them belonged to the GFD group and 52.6% to the RD group. There was no significant difference between the two groups (P=0.81).

The gastrointestinal status, based on the ROME III questionnaire, of the two groups is shown in Fig. 1. Paired t test revealed a significant decrease (57.56%) in the mean score of gastrointestinal status for the GFD group (P<0.001). Conversely, the RD group posted gains (6.74%) on the mean score of gastrointestinal status, and no significant result was observed (P=0.40).

The results of the gastrointestinal measures based on the ROM III questionnaire are shown in Table 2. The McNemar test revealed that there were significant differences before and after the GFD in the GFD group for stomach ache (P=0.04) and bloating (P=0.005). Conversely, there were no significant differences in the RD group.

Wilcoxon's test demonstrated that there were significant differences in constipation and diarrhea before and after the intervention in the GFD group (P<0.001). However, there were no significant differences in the RD group (P>0.999).

Behavioral outcomes

The behavioral measures of the two groups are shown in Table 3. There were significant differences in stereotyped behaviors, communication and social interaction before and after the GFD intervention in the GFD group (P<0.05). However, there were no significant differences in the RD group (P>0.05).

The subclasses in stereotyped behaviors and social interaction showed a significant decrease in the GFD group (P<0.05) (Table 4). Fig. 2 illustrates the differences in stereotyped behaviors, communication and social interaction between the two groups. ANCOVA revealed

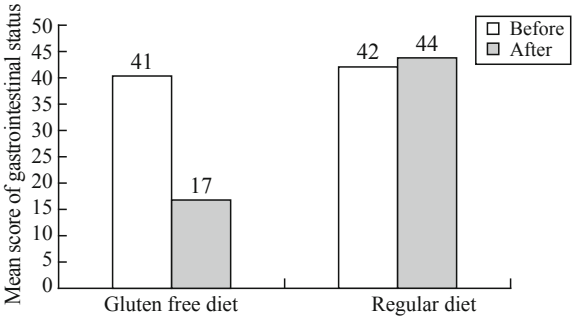

Fig. 1. Comparison of gastrointestinal scores before and after intervention in the study groups.

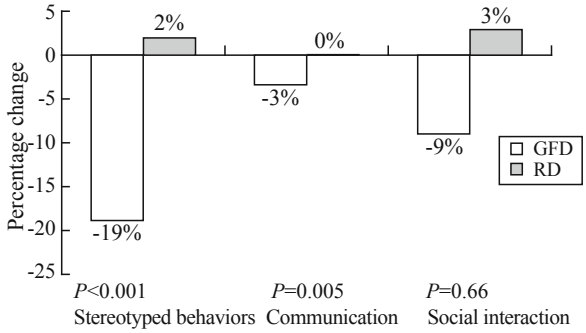

Fig. 2. Percentage change of differences in stereotyped behaviors, communication and social interaction between the study groups at baseline and follow-up. GFD: gluten free diet; RD: regular diet.

Table 2. Comparison of gastrointestinal symptoms between groups at baseline and fallow-up

Variables	Gluten free diet (n=38)		P value	Regular diet (n=38)		P-value
	Baseline	6 wk		Baseline	6 wk	
Stomachache, n (%)	11 (28.9)	7 (18.4)	0.04	10 (26.3)	12 (31.6)	0.16
Bloating, n (%)	21 (55.3)	13 (34.2	0.005	18 (47.4)	19 (50)	0.56
Constipation, n (%)	19 (50.0)	2 (5.3)	<0.001	14 (36.8)	18 (47.4)	0.046
Diarrhea, n (%)	1 (2.6)	1 (2.6)	1.00	3 (7.9)	5 (13.2)	0.31

Categorical variables were analyzed by Wilcoxon's rank-sum test and/or independent t test.

Table 3. Comparison of behavioral measures, between groups at baseline and follow-up

Variables	Gluten free diet (n=38)		P value	Regular diet (n=38)		P value
	Baseline	6 wk		Baseline	6 wk	
Stereotyped behaviors, mean±SD	17.61±6.74	14.5±7.92	<0.001*	18.92±5.89	19.32±6.27	0.25*
Communication, mean±SD	33.87±8.31	32.97±9.28	0.017†	32.53±9.48	32.58±9.40	0.81†
Social interaction, mean±SD	19.00±50.00	2.00±5.30	<0.001*	14.00±36.80	18.00±47.40	0.046*
Total score, mean±SD	18.14±19.36	14.91±16.94	0.002†	18.99±21.50	20.72±22.72	0.054†
Autism index, mean±SD	80.03±14.07	75.82±15.37	0.001*	79.92±15.49	80.92±16.24	0.12*

SD: standard deviation. *: paired t test; †: Wilcoxon's rank-sum test.

Table 4. Items of the subclasses in GARS-2 questionnaire

Subclass	Item	*P* value
Stereotyped behaviors	Stares at hands or objects for 5 seconds	0.031
	Eats special foods and does not like foods that most people eat	0.031
	Smells or sniffs objects	0.039
	Turns around self or moves in a special rhythm	0.022
	Sudden movements	0.012
Social interaction	Avoids eye-contact	0.016
	Repetitive stereotyped behaviors	<0.001
	Negative and aggressive behavior to orders	0.031

Variables were analyzed by Signed test.

that there were significant decreases in stereotyped behaviors, communication and social interaction in a subgroup of children with ASD after adjustment of preliminary variables.

Pearson's product-moment correlation coefficient method showed that there were no significant correlations between behavioral, gastrointestinal and serological measures before and after the GFD intervention ($P<0.05$).

Discussion

In the past years, studies[16,22-26] have been conducted to identify the association between ASD and gluten, attempting to determine the specificity of antigens involved in celiac disease and also their pathogenic connection with ASD. In different studies, the exact percentage of children with ASD who suffer from gastrointestinal diseases varies, but these studies agree that gastrointestinal abnormalities are common in ASD.[18] Jyonouchi[32] reported that young children are more sensitive to certain food proteins because of the immature gut mucosal immune system. This might explain the high incidence of gastrointestinal abnormalities in our study. Additionally, bloating and constipation as the most prevalent symptoms occurred in 21% and 19% of children respectively. Similarly, Levy and colleagues[33] reported that the incidence of gastrointestinal symptoms was 54%. In our study, the incidence of gastrointestinal symptoms in children decreased significantly (57.56%). The high incidence of gastrointestinal symptoms in children with ASD is related to food allergies.[32] The reduced incidence of gastrointestinal symptoms in our study may be due to the elimination of gluten. Moreover, there is a strong evidence that gastrointestinal symptoms are in association with aggressive behavior patterns in children with ASD.[34] Therefore, the reduced incidence of gastrointestinal symptoms in our study may be attributable to the mitigated aggressive behaviors.

Studies[5,35] suggested the reduction of ASD behavioral patterns and improvement of developmental outcomes in at least some children taking gluten free diet, casein free diet, or gluten and casein free diet. Mari-Bauset et al[19] recommended gluten and casein free diet for children who were diagnosed with intolerance or allergies to gluten and/or casein. There is no enough evidence to support gluten and casein free diet.[36,37] Harris and Card[12] reported the improvement of gastrointestinal symptoms and behavior patterns in children on a gluten and casein free diet. However, the Gastrointestinal Symptoms Rating Scale (GSRS) and Childhood Autism Rating Scale (CARS) scores did not support this association.

In contrast to the gastrointestinal symptoms, autistic behaviors (5.32%), stereotyped behaviors (18.97%), communication (3.45%) and social interaction (9.15%) had slight improvements. Mild improvement in communication perhaps was due to the short period of diet intervention. However, Seung et al[38] reported that there was no significant difference in oral and nonverbal communication between gluten and casein free diet and regular diet. Another study[39] found that gluten and casein free diet for children with gastrointestinal symptoms, food allergy, and suspected food sensitivity was effective in improving ASD behaviors, physiological symptoms, and social behaviors compared with those without these abnormalities. Additionally, it was found that gluten and casein free diet should be given for children with acute behavioral changes and/or those with allergies or food intolerances to gluten or casein.[18] Mulloy[40] also found that the gluten and casein free diet was not effective in the treatment of the core symptoms of ASD, i.e. social and communication deficits and stereotypic/repetitive behaviors. Studies excluding milk from children's diet also revealed the improvement of autistic behaviors.[41] In our study, the results of GARS-2 questionnaire showed no correlation between gastrointestinal total score and autism severity ($P=0.599$). On the contrary, Adams et al[11] found that the results of six-item Gastrointestinal Severity Index (6-GSI) questionnaire were significantly correlated with those of the total Autism Treatment Evaluation Checklist (ATEC) ($r=0.60$, $P<0.001$). Also, there was a strong correlation of gastrointestinal symptoms with autism, which indicates that children with complex autism may have more complicated gastrointestinal symptoms.

Our study has some limitations. First, the small sample size and heterogeneity of the patients limited the results of the study. However, the results of subgroups of patients indicated that children aged 4-8 years showed a significant reduction in gastrointestinal symptoms ($P<0.001$) and autistic behaviors ($P=0.002$). Furthermore, the short duration of the intervention was another major limitation to this study. Second, parents of the patients were not blinded to the

intervention, which influenced their awareness of children's behaviors. In contrast, there is a chance of over reporting, i.e. parents might have declared positive effects when the effects may not have been present or not as prominent as they reported. Furthermore, for limited education, low economic income and lack of knowledge and understanding of autistic behaviors, parental cooperation was weak. Third, even though parents tried to hide prohibited foods and were careful about food sneaking, they were not always successful. Therefore, it would have been superior, replicating the study in a more supervised setting and lowering the possibility of diet disruption.

Moreover, excluding wheat as one of the important, inexpensive and accessible cereals of developing eastern diet and specially children with ASD who are delicate to the smell, structure and taste of foods is also challenging.[10,34] Children with ASD often have special food cravings and refuse to eat particular foods. Therefore, persistence on an elimination diet can be challenging.[34] Finally, psychiatric illnesses especially ASD are influenced by seasonal changes and circadian rhythms. Changes in behavior and cognitive functioning have been reported in these cases.[16]

In conclusion, this small randomized clinical trial indicated that ASD has a heterogeneous spectral nature and that it seems unlikely that everyone would benefit from the gluten free diet.[5,35] Our findings support for the use of the gluten free diet in the treatment of some children with ASD, and stress the importance of further research into the biological and dietary factors optimizing and attenuating the diet in treating these children. Additionally, searching for ASD subpopulations that will best respond to the intervention is another aspect that must be taken into account.

Acknowledgements

The authors appreciate the financial support of the Nutrition Research Center, Tabriz University of Medical Sciences and Iranian Special Education Organization for children with pervasive developmental disorders and the patients who participated in this study.

Funding: Supported by Nutrition Research Center, Tabriz University of Medical Sciences.
Ethical approval: Ethics Committee of Tabriz University of Medical Sciences and was registered in the Iranian Registry of Clinical Trials website (IRCT201404212017N20).
Competing interest: The authors declare that there is no conflict of interest.
Contributors: Faezeh Ghalichi proposed the project and wrote the paper. Faezeh Ghalichi is the guarantor.

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Received October 21, 2014
Accepted after revision January 22, 2015