ORIGINAL ARTICLE

# The Effect of Gluten-Free Diet on Gastrointestinal Symptoms and Disease Severity of Autistic Children: A Randomized Controlled Trial

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## ABSTRACT

**Objectives:** Autism Sspectrum Disorder (ASD) is a kind of neurodevelopmental disease characterized by difficulties in social interactions, verbal and non-verbal communication, movement limitations, and repetitive movement patterns. The goal of this study is to investigate the effects of a Gluten-Free Diet (GFD) on gastrointestinal (GI) and neurological symptoms in patients diagnosed with autism.

**Materials & Methods:** In this study, 120 patients with autism from Tabriz Children's Hospital and Sheikh Al-Rais Clinic were included. Neurologists and psychologists confirmed the diagnosis of autism using the M-CHAT-R/F questionnaire. Based on the patients' clinical history and Rome 3 criteria, GI symptoms such as diarrhea, constipation, vomiting, and abdominal discomfort were detected. A Gluten Free Diet was advised, and the parents received nutrition education and ongoing autism therapies. The control group consisted of children who received specialized medications for autism while maintaining a regular diet. These children were monitored closely.

**Results:** The average age of the patients was 9.27 ± 3.25 years with a median of nine years. Fifty-two patients (47.3%) were boys, and fifty-eight patients (52.7%) were girls. The severity of ASD, as measured by the M-CHAT-R/F scale, was significantly reduced at the 12th month in the intervention group. Furthermore, significant improvements in speech, cognition, and behavior have been observed in patients in the intervention group after using a GFD. Moreover, GI symptoms, including nausea and vomiting, constipation, abdominal pain, and discomfort, were significantly decreased in the intervention group. However, the GI symptoms in the control group did not show any statistically significant difference compared to the 12th month.

**Conclusion:** According to the results obtained in this study, the administration of GFD in children with ASD can significantly lead to the improvement of GI disorders and neurological symptoms regarding the severity of autism in speech, cognition, and behavior.

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## Introduction

Autism Spectrum Disorder (ASD) is one of the neurodevelopmental disorders characterized by difficulties in social interactions, verbal and non-verbal communication, along with movement and interest restrictions, and repeated patterns of activity (1). According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), the main characteristics of ASD include persistent social communication and interaction limitations with restricted and repetitive behaviors, activities, and interests (2). In addition, autistic children frequently

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display serious behavioral disturbances, such as aggression, irritability, and self-injurious behavior (3, 4). Autism typically emerges before the age of three, and its main cause is unknown (5). Families and caregivers of children diagnosed with autism experience a vast amount of difficulties; one of the main challenges for them is establishing an effective relationship with the autistic children (6), as the main symptom of ASD is limitation in social communication, even sometimes detecting dangerous behaviors of these patients (7). By researching the human body's immune system, genetic capabilities, and environmental factors such as nutrition, infections, and vaccines, various theories have been put forth regarding the biological etiology of autism. On the other hand, according to these theories, parents of these children may sometimes use unapproved treatment methods to take care of and manage their autistic children (8). Nutritional therapy in children is not a new topic and has been discussed in various studies since the 1920s (9). Feingold's 1970s study stands out as the most widely cited research on this topic. It reported that approximately 50% of children experiencing symptoms of hyperactivity and intellectual challenges showed significant improvement when they followed a diet that restricted salicylates (10). In studies conducted in the 1980s, it was reported that diets with high sugar content have a negative effect on symptoms of hyperactivity and aggressiveness (11). In the study conducted by Cade et al. on schizophrenia, Dohan's hypothesis was investigated. According to this hypothesis, schizophrenia is sometimes associated with the absorption of the "Exorphin" substance found in "Gluten" and "Casein" (12). Gluten is a mixture of proteins found in various grains, such as oats, barley, and wheat. Gluten is a combination of two glycoproteins, "Gliadin" and "Glutenin", which are bound to glucose in the endosperm (the nutritional layer surrounding the seeds) of many cereal grasses (13).

A Gluten-Free Diet (GFD) is one of the alternative treatments for children with autism. If this diet is followed, all foods containing gluten, such as wheat, barley, and oats, must be removed from the child's daily diet (14). In recent years, the GFD has become one of the most popular diets among the general public and patients who suffer from several clinical conditions, such as autism, Irritable Bowel Syndrome (IBS), Non-Celiac Gluten Sensitivity (NCGS), as well as rheumatologic, neurological, and psychiatric diseases, as well as for the enhancing performance of sports practice (15). Recent studies have reported that the use of gluten and casein-restricted diets in ASD patients may lead to associated improvements in symptoms, resulting in enhanced language abilities and better social communication. However, to our knowledge, only a few clinical trials have been conducted on this topic, and these findings have not been scientifically validated. Even though ASD is mostly defined by neurological, social, and behavioral symptoms, various comorbidities are frequent in these children (16). Moreover, individuals with ASD are more likely to have co-existing gastrointestinal (GI) symptoms, such as nausea, vomiting, constipation, diarrhea, and abdominal pain, but these symptoms are in a less attention-grabbing manner (17). Due to the limitations in the ability of autistic children for verbal communication and expressing their different perception of pain, the diagnosis and understanding of internal symptoms such as heartburn, pain, discomfort, nausea, vomiting, and others, are often very challenging (18). Thus, the onset of GI diseases in children with autism sometimes occurs with a delay, and the diagnosis includes more difficulties compared to healthy children (19, 20). One of the high-priority goals in caring for an autistic child's diet, as with any other child, is to provide the nutrients their body needs to maintain their health throughout their life. Children with autism and their families should collaborate with a special care team, including a nutrition specialist. The team should evaluate the quality of the nutrients in the child's diet, recognizing that nutrient deficiencies may persist even when the child has appropriate growth (21). Determining the efficacy of various diets for autistic children can provide a basis for identifying the appropriate special diet for these children as soon as possible, which may lead to better treatment outcomes for these patients (22). Therefore, this study aims to determine the effect of following a GFD on GI and neurological symptoms in children diagnosed with autism. This study evaluated the results of 110 children to assess the effects of GFD on neurological and GI symptoms of children diagnosed with ASD.

## Materials & Methods

### *Participants*

This study is a randomized controlled trial with target patients aged between 2.5 and 16 years old diagnosed with ASD and accompanying GI symptoms. Participants were randomly assigned to either the intervention group (GFD) or the control group (regular diet) using a computer-generated randomization list. The inclusion criteria included an ASD diagnosis confirmed by the M-CHAT-R/F questionnaire (23) and the presence of GI symptoms such as constipation, diarrhea, vomiting, and abdominal pain. Patients with parental dissatisfaction with compliance with the gluten-free treatment regimen, those diagnosed with a

specific GI disease, or those who aged out of the defined range were excluded from the study.

***Measures***

In this study, all patients with autism referred to the Sheikh Al-Rais Clinic and Tabriz Children's Hospital were enrolled. Patient demographic information was recorded on a data collection form. Each form had a specific code. Furthermore, demographic information for patients, including age, gender, type of GI symptoms, improvement status, and any changes in neurological and GI symptoms after prescribing GFD, was included. The M-CHAT-R/F and Autism Treatment Evaluation Test (ATEC) questionnaires were used to evaluate the ASD status of the patients. Patients were followed up for ASD and treatment response at baseline and at the 12th month follow-up.

***M-CHAT-R/F questionnaire***

The M-CHAT-R/F, first introduced in 1999, is an updated version of the original M-CHAT (Modified Checklist for Autism in Toddlers) designed to screen for early signs of autism in children. If a child scored between 8 and 20, they were classified as high risk for autism and referred immediately for more detailed diagnostic and therapeutic interventions, as well as additional neurological and GI evaluations, without the need for further M-CHAT-R/F screening. If the child is under two years old and scores between 0 and 2, the risk is considered low. A complete medical history and physical examination were performed, and there was no need for follow-up regarding ASD for these patients. Patients with scores between 3 and 7 were considered to have average risk, and the M-CHAT-R/F questionnaire was administered at subsequent follow-ups visits.

***ATEC questionnaire***

The ATEC questionnaire (24) was used to evaluate various aspects of patient neurological signs and symptoms. The main purpose of this questionnaire was to evaluate treatment response and changes in ASD status during treatment. This questionnaire had four domains: Language, speech, and communication (14 items); sensory and cognitive awareness (18 items); sociability (18 items); behavior and physical health (25 items). Each item received a score of 0 to 2 in the first three domains and 0 to 3 in the last domain. The total score of the questionnaire also ranged from 0 to 175.

***Rome III criteria***

GI symptoms were evaluated at the beginning of the study. A standard blood test including CBC diff, ESR, occult blood, and S/E stool tests, were performed for all patients. In cases of reported abdominal pain, an abdominal ultrasound and U/A were performed. These measures were taken to identify patients with GI symptoms other than autism so that patients with a specific GI diagnosis could be excluded from the study. A complete physical examination and pain assessment were performed using a specific pain index, the Verbal Rating Scale of Pain (VRS). GI symptoms, including diarrhea, constipation, vomiting, and abdominal pain, were defined based on the patients' medical history and Rome III criteria (25). The pattern of bowel movements and evidence of malabsorption, including bulky and fatty stools, were confirmed based on the parents' reports, and a pediatric gastroenterology specialist evaluated the symptoms. The patients' GI symptoms were followed up at baseline, 6 months, and 12 months.

***Intervention***

In addition to ongoing autism-related treatments, children in the intervention group were prescribed a strict GFD, which eliminated all gluten-containing foods, including wheat, barley, rye, and oats. The diet included gluten-free alternatives such as rice, corn, potatoes, and various legumes. The children's parents received detailed nutritional education on identifying gluten-containing foods. Special attention was given to ensuring that the children did not experience any nutrient deficiencies, such as those in fiber or B vitamins, which are common in a GFD. The adherence to the GFD was monitored through parents' food diaries and regular follow-up consultations to discuss diet-related challenges and assess potential side effects. The control group consisted of children who received only standard medications for autism but maintained their regular diet. The final status and response to the dietary regimen were compared between the two groups.

***Ethical Considerations***

The study obtained ethical approval from the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1400.089). Before completing the patient information questionnaire, the patient's parents were fully informed of the plan and the reasons for its implementation. Informed consent was obtained from the parents or legal guardians of participants. The children's parents were informed that they are free to leave the trial at any time.

***Statistical data analysis***

The data were analyzed using the SPSS software (version 23). The normality of the data was checked using the Kolmogorov – Smirnov test. Descriptive statistics were presented as frequencies (percentages)

for qualitative variables, mean ± standard deviation for normally distributed quantitative variables, and median for non-normally distributed quantitative variables. Comparisons between baseline and follow-up measurements within each group were performed using a paired t-test. Between-group comparisons were conducted using the independent t-test, Mann-Whitney U test, or chi-square test, as appropriate. For categorical outcomes measured at multiple time points, Cochran's Q test was used to assess changes over time. In this study, a type I error rate of 5% ($\alpha = 0.05$) was used as the criterion for statistical significance in analyses.

## Results

In this study, 120 patients diagnosed with ASD accompanied by GI symptoms like abdominal pain, nausea, vomiting, constipation, and diarrhea were investigated. Patients were randomly categorized into two groups: Control and Intervention. Fig 1 illustrates the flowchart for patient selection to enter the investigation. Ultimately, 54 patients in the intervention group and 56 patients in the control group were evaluated.

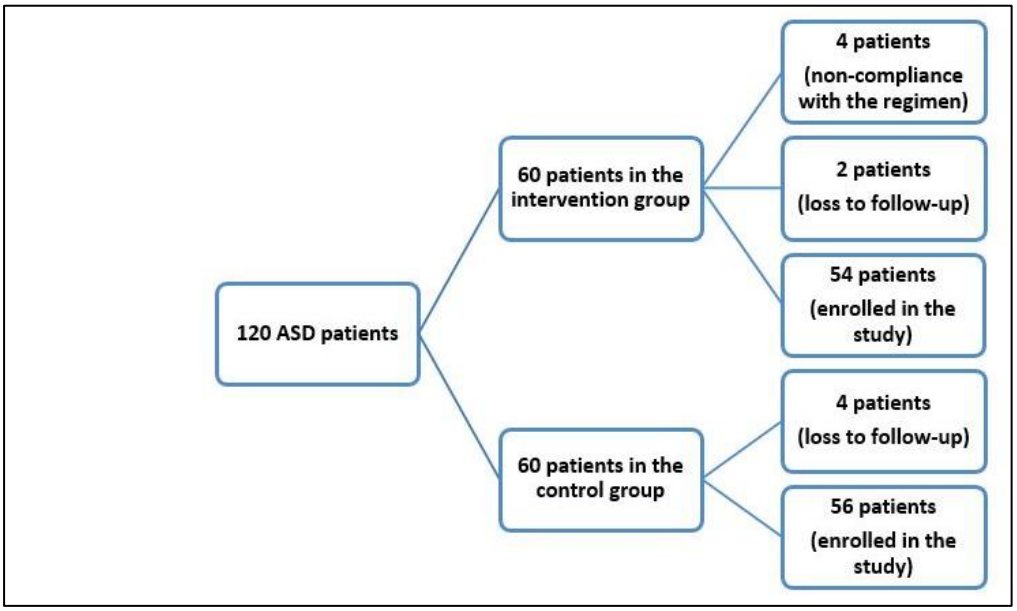


**Figure 1:** The flowchart for patients entry in the study

The average age of the patients was 25.3 ± 27.9 years, with a median of nine years. The youngest patient in the trial was six years old, and the oldest was 16. Demographic information of patients by study groups is presented in Table 1. Based on the results, no significant difference was found between the intervention and control groups in age or gender distribution, and the patients in both groups were homogeneous regarding demographic variables.

**Table 1.** The Demographic data of the patients

| Variable | | Intervention group (n=54) | Control group (n=56) | p-value |
|---|---|---|---|---|
| **Age (y), (mean±SD)** | | 9.14 ± 3.32 | 9.38 ± 3.22 | 0.465 |
| **Sex, n(%)** | **Male** | 23 (42.6%) | 29 (51.8%) | 0.219 |
| | **Female** | 31 (57.4%) | 27 (48.2%) | |

The severity of ASD was evaluated based on the M-CHAT-R questionnaire. The patients were assessed for ASD severity at the beginning of the study and 12 months after the intervention. The results are presented in Table 2. The severity of ASD, as measured by the M-CHAT-R/F scale, decreased significantly in the intervention group at the 12-month endpoint. However, no statistically significant difference in ASD symptoms severity was observed in the control group between baseline and 12 months after the beginning of the study. Likewise, the classification of autism severity in the study patients at baseline and 12 months later is shown in Table 2. Based on the results, the number of cases with moderate and severe autism in the intervention group decreased significantly after the dietary intervention, whereas, no significant difference was observed in the control group.

The analysis of adherence to GFD according to neurological status is shown in Table 3. No significant difference in ATEC score was observed between the baseline and 12-month follow-up periods for the control group (p = 0.903). In contrast, a significant reduction in the ATEC score was observed at 12-month follow-up in the intervention group (p = 0.001). Significant gains from the intervention were reported for speech, cognition, and behavior, but not for sociability. The control group showed no difference in scores across any of the subscales. Overall, the decrease in ATEC scores was greater in the intervention group, indicating a considerable improvement in neurological status (particularly speech, cognition, and behavior).

**Table 2.** The evaluation of ASD symptoms severity in the studied participants regarding the M-CHAT-R/F questionnaire and severity categories

| M-CHAT-R/F Scores | | | | |
|---|---|---|---|---|
| **Time Point** | | **Intervention group (mean ± SD)** | **Control group (mean ± SD)** | **p-value** |
| Baseline | | 7.52 ± 2.63 | 6.63 ± 3.20 | **0.263** |
| 12th month | | 4.12 ± 0.89 | 6.05 ± 2.84 | **0.003*** |
| Rate of change | | -3.39 | -0.58 | |
| p-value | | **0.001*** | 0.419 | |
| **ASD Severity Classification** | | | | |
| **Time** | **Severity Category** | **Intervention Group n (%)** | **Control Group n (%)** | **p-value** |
| Baseline | Mild symptoms | 7 (12.6%) | 6 (11.2%) | |
| | Moderate symptoms | 40 (71.4%) | 38 (70.3%) | **0.409** |
| | Severe symptoms | 9 (16%) | 10 (18.5%) | |
| 12th month | Mild symptoms | 23 (42.6%) | 8 (14.2%) | |
| | Moderate symptoms | 25 (46.3%) | 38 (67.9%) | **0.003*** |
| | Severe symptoms | 6 (11.1%) | 10 (17.9%) | |

**Table 3.** ATEC Outcomes

| ATEC Domain | | Intervention group (mean ± SD) | Control group (mean ± SD) | p-value |
|---|---|---|---|---|
| **Total Score** | Baseline | 64.13 ± 17.77 | 62.82 ± 18.14 | |
| | 12th month | 42.13 ± 15.82 | 61.60 ± 16.17 | **0.002*** |
| | Rate of change | -18.49 | 0.74 | |
| **Speech** | Baseline | 21.20 ± 8.14 | 22.93 ± 11.54 | |
| | 12th month | 15.47 ± 3.78 | 23.00 ± 11.33 | **0.001*** |
| | Rate of change | -20.99 | 0.79 | |
| **Sociability** | Baseline | 17.53 ± 4.41 | 17.87 ± 2.80 | |
| | 12th month | 16.73 ± 4.17 | 17.93 ± 2.43 | **0.539** |
| | Rate of change | -0.46 | 1.12 | |
| **Cognition** | Baseline | 18.33 ± 7.00 | 19.33 ± 6.77 | |
| | 12th month | 15.07 ± 5.41 | 19.40 ± 6.95 | **0.002*** |
| | Rate of change | -10.87 | 0.11 | |
| **Behavior** | Baseline | 16.53 ± 6.73 | 13.73 ± 4.17 | |
| | 12th month | 11.80 ± 4.78 | 13.72 ± 4.18 | **0.001*** |
| | Rate of change | -22.26 | 0.00 | |

GI symptoms were evaluated in four categories: Nausea and vomiting, constipation, abdominal pain and discomfort, and diarrhea. A comparison between control and intervention groups at baseline, 6 months, and 12 months is presented in Table 4. The intervention group reported reduced nausea and vomiting compared to the control group at both 6 months and 12 months. The incidence of constipation was also significantly lower in the intervention group at 6 and 12 months. An improvement was observed in the intervention group, but not in the control group. In addition, improvements in abdominal pain and discomfort in the intervention group relative to the control group were statistically significant at 6 and 12 months. The intervention group showed a remarkable change in abdominal pain over time, whereas the control group showed no significant change. In addition, diarrhea was significantly reduced in the intervention group at 12 months compared to the control group. The intervention group exhibited a reduction in diarrhea cases; however, no significant changes were observed within the control group.

## Discussion

ASD is one of the neurological developmental disorders that manifests as difficulties in social interaction, verbal and nonverbal communication, along with motor deficits, as well as restrictive and repetitive movement patterns (26). Families of children

with autism seek supplementary supportive treatments, along with clinical interventions for the disease, including nutritional therapies, specifically effective dietary restrictions (27, 28). Many children with ASD also suffer from GI symptoms, such as abdominal pain, constipation, diarrhea, nausea, vomiting, and bloating (29). Some researchers have hypothesized that the link between GI symptoms and ASD may be related to gluten sensitivity or celiac disease, an autoimmune disorder caused by an immune reaction to gluten (30). Therefore, one theory is that a GFD may help alleviate these symptoms and improve overall health outcomes of individuals with ASD.

**Table 4.** Gastrointestinal Symptom Frequency: Control vs. Intervention (Baseline, 6 Months, and 12 Months)

| GI Symptom | Time points | Intervention group, n(%) | Control group, n(%) | p-value (Between-Group) | p-value (Within Intervention Group) | p-value (Within Control Group) |
|---|---|---|---|---|---|---|
| **nausea and vomiting** | Baseline | 9 (16.7%) | 14 (25%) | 0.408 | | |
| | 6th month | 6 (11.1%) | 13 (23.2%) | **0.009*** | **<0.001*** | 0.898 |
| | 12th month | 4 (7.4%) | 14 (25%) | **0.001*** | | |
| **constipation** | Baseline | 9 (16.7%) | 12 (21.4%) | 0.309 | | |
| | 6th month | 7 (12.9%) | 12 (21.4%) | **0.019*** | **<0.001*** | 0.409 |
| | 12th month | 4 (7.4%) | 11 (19.7%) | **0.008*** | | |
| **abdominal pain and discomfort** | Baseline | 20 (37%) | 18 (32.1%) | 0.309 | | |
| | 6th month | 8 (14.8%) | 16 (28.6%) | **0.001*** | **<0.001*** | 0.907 |
| | 12th month | 4 (7.4%) | 17 (30.4%) | **0.001*** | | |
| **diarrhea** | Baseline | 12 (22.3%) | 13 (23.2%) | 0.571 | | |
| | 6th month | 7 (12.9%) | 12 (21.4%) | **0.001*** | **<0.001*** | 0.556 |
| | 12th month | 5 (9.3%) | 14 (25%) | **0.001*** | | |

The concept of GFD for individuals with ASD is not new. It has been proposed since the 1990s as a possible intervention for some individuals with ASD who also have GI symptoms (31, 32). Gluten is a type of protein found in wheat, barley, and rye, and some people may be sensitive or intolerant to it. In recent years, there has been an increase in the overall number of children diagnosed with autism who are following a GFD, either on their own or at the recommendation of their parents or healthcare provider. However, some studies have also shown that in some patients with autism, behavioral disorders have improved following dietary restrictions, and after reintroducing the same foods, their behavioral disorders have worsened (33, 34). For example, in some studies, gluten-free and casein-free diets have led to significant improvement in neurological symptoms in patients with autism. In contrast, in other studies, these diets did not affect patients' behavioral status (35, 36). The present study was designed and conducted to investigate the effect of a GFD on GI and neurological symptoms in patients with autism.

The current study was conducted on 110 patients manifesting ASD features along with GI symptoms. Pediatric neurology subspecialists and psychiatrists confirmed the diagnosis, and the patients were divided into two groups: A control and an intervention group. The intervention involved as much as possible setting up gluten-free foods in the child's diet, while the control group received their regular family diet. The children's parents were educated about the intervention. The patients' names were kept private, and essential ethical considerations were taken into account. Moreover, the intervention focused on limiting the gluten-containing foods in the child's diet, so there was no need to prepare special packages from the market. GI experts do not fully confirm the effectiveness or potential side effects of these packages. Thus, this study was cost-effective and safe, without imposing any additional costs on families or causing any vitamin or nutritional deficiencies in the child's diet. Finally, the patients were followed for 12 months regarding neurological and GI symptoms. The results of the current study showed that neurological symptoms in patients significantly decreased after starting the intervention, as measured by the M-CHAT-RF questionnaire, among those who received a GFD. Therefore, gluten restriction had a significant effect on patients' neurological symptoms over a 12-month period. Based on the ATEC questionnaire, significant improvements were also observed in speech, cognition, and behavior in patients of the intervention group after using a GFD.

Consistent with this study, a review by Whiteley et al. reported that in follow-up studies using the ATEC questionnaire, 20-29% of patients treated with GFD showed significant improvement in ASD-ralated neurological symptoms (37). Moreover, Piwowarczyk et al. utilized the Autism Diagnostic Observation Schedule-Second Edition (ADOS-2) checklist to evaluate the effect of a GFD on the neurological status of patients with ASD. However, no difference was

found between the gluten-free and regular diet groups in terms of the ASD symptoms (38).

In a study by Gonzalez-Domenech et al., the behavioral changes were investigated using the ATEC questionnaire. Nevertheless, no behavioral changes were observed six months after starting a gluten- and casein-free diet. One reason could be the short follow-up time and non-compliance with the diet in some patients, which might lead to bias and explain the lack of agreement with the current study (39). Stewart et al. reported that children with autism who require a gluten-free and casein-free diet should be prescribed dietary supplements containing calcium, potassium, vitamin D, and choline (40). Furthermore, Mari-Bauset et al. showed that that fiber, vitamin, and vegetable intake in patients on a gluten- and casein-free diet was better than in the control group. However, no difference was observed between disease status (41). In a randomized controlled trial study conducted in 2016 by Ghalichi et al., the severity of GI symptoms and behavioral disorders was significantly reduced in ASD patients who received GFD, from 40.57% to 17.1% for GI symptoms and from 80.03% to 75.82% for behavioral disorders (42). Piwowarczyk et al. reviewed relevant clinical trials and concluded some significant improvement in social communication among patients who received a gluten- and casein-free diet (43).

Moreover, in the present study, the evaluation of GI symptoms, including nausea and vomiting, constipation, abdominal pain and discomfort, and diarrhea, showed a significant reduction in the intervention group. Specifically, a 55.6% decrease in nausea, vomiting and constipation, an 80% decrease in abdominal pain and discomfort, and a 58.3% decrease in diarrhea were observed. In contrast, statistically significant difference in GI symptoms was found in the control group between baseline and 12 months later. Therefore, it can be concluded that gluten restriction in patients with ASD can significantly improve GI symptoms over a 12-month period. GI symptoms are more common in children with ASD compared to healthy children (44). The prevalence of GI symptoms is widely variable, ranging from 23% to 70%, including constipation, diarrhea, vomiting, abdominal pain, reflux, and bloating (45, 46). The severity of GI symptoms is also directly correlated with the severity of autism (47). Bidirectional changes in the brain-gut microbiome have been identified as the main pathophysiology of brain-related disorders such as autism, Parkinson's disease, and chronic pain. However, the exact mechanisms underlying these changes remain unclear (48-50).

Although it is predicted that a therapeutic diet can improve gut microbiota and reduce toxin production, studies to date have not reported definitive results in this regard (51, 52). However, some studies have shown that the use of these diets can improve the symptoms of individuals with ASD, and a gluten-free and casein-free diet is one such regimen that is commonly used in these patients (53). This regimen was first introduced in the 1980s based on the idea that the presence of gluten and casein in food could cause autism-like symptoms due to changes in brain function (54). Studies have reported that undigested protein molecules may pass through intestinal mucus in children with ASD, a phenomenon attributed to immune factors or intestinal lesions (55). Eventually, these peptides enter the bloodstream and pass through the blood-brain barrier, leading to negative effects on the brain, including difficulties with concentration, normal brain development, social interaction, and learning (56). In a study on rats, it was observed that a direct relationship exists between urine protein patterns and abnormal brain chemistry (57). Recent studies have also reported that peptides and proteins related to them are different in the urine of ASD patients compared to healthy individuals (58). Additionally, another study found that the urinary protein pattern changed after following a GFD (59).

Knivsberg et al. reported that a gluten-free and casein-free diet can improve behavioral symptoms, nonverbal cognition, and motor problems in patients, consistent with the current study (31). Conversely, in contrast to the current study, Cass et al. did not observe any improvement in neurological symptoms following the prescription of a gluten-free and casein-free diet (60). Furthermore, in Elder's study, after a 6-month follow-up, no changes in urinary protein patterns or neurological symptoms were observed in response to the prescribed diet, unlike in the current study (61). Winburn et al. reported that in children with ASD treated with a gluten-free and casein-free diet, at least one GI symptom improved, and some patients also showed increased attention and decreased repetitive behaviors related to the disease, findings consistent with the current study (62). On the other hand, a study by Pusponegoro et al. reported no significant changes in GI, and neurological symptoms after restricting gluten and casein in the patients' diet (63). However, the result obtained in this study seems to be due to the short duration of the diet, as studies have suggested that the effectiveness of a GFD requires at least six months (64).

This study had some limitations. First, the sample size is small, and the study has a single-center nature, making it difficult to generalize the results to the general population. Second, the intervention was not hidden from the patients' parents, which might have

affected their knowledge of their children's behavior. Besides, one of the main differences between the current study and others is the intervention method. In the present study, the intervention involved the family reducing gluten-containing foods as much as possible whereas in other relevant studies, both gluten and casein were restricted in the child's diet. Nevertheless, using this diet imposes high costs on families. Despite this issue, significant improvements in GI and neurological symptoms were observed in the current study.

## In Conclusion

This study concluded that reducing gluten-containing foods in the diets of children with ASD can significantly improve gastrointestinal and neurological symptoms related to autism severity, particularly in speech, cognition, and behavior. However, notably, studies on the effects of therapeutic diets in patients with ASD are still limited, with mostly small sample sizes, and the reported results in this area are sometimes contradictory and heterogeneous. Further research is demanded to determine the long-term effects of GFD and other specific diets on children with ASD, as well as to investigate their effects on adults with ASD. We advise investigating larger populations in future studies and giving more careful consideration to neurological and GI symptoms in patients.

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***Authors' Contribution***

M.S: Conceptualization of the study, study design, drafting and revising the manuscript.

P.G: Data collection, statistical analysis, and drafting and revising the manuscript.

A.D.A: Study design, drafting, and revising the manuscript.

S.S: Supervision, critical revisions to the manuscript.

M.V: Literature review, assisted with data collection, and revised the manuscript for intellectual content.

A.A: Data collection, interpretation of results, and revising the manuscript for accuracy.

***Conflict of Interest***

The authors declare no conflict of interest.

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