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EFFECT OF GLUTEN-FREE, CASEIN-FREE DIET, SUPPLEMENTS AND DIGESTIVE ENZYMES ON GASTROINTESTINAL SYMPTOMS AND BEHAVIOR IN INDIVIDUALS WITH AUTISM SPECTRUM DISORDER

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ABSTRACT

Autism Spectrum Disorder (ASD) presents as a complex neurodevelopmental condition, encompassing various symptoms, including gastrointestinal (GI) issues. This study explores the impact of a gluten-free, casein-free diet (GF/CFD), complemented by digestive enzymes and nutritional supplements, on GI symptoms and behavior in individuals diagnosed with ASD. The study includes 70 participants, with 34 in the treated group and 36 in the non-treated group, spanning a 6-month duration. The investigation evaluates changes in GI symptoms, behavioral measures, and co-occurring psychopathological conditions.

Results reveal significant improvements in numerous GI symptoms, such as abdominal discomfort, bloating, constipation, hard stool, rumbling, and flatus, among others, following the dietary intervention. Notably, these positive changes persist and deepen over time. Additionally, certain behavioral aspects, including play skills, sociability, eye contact, sleep, stools/gut issues, display substantial enhancements in the treated group compared to the non-treated group. Language and cognition/thinking exhibit not significant improvements. The total score of the treatment group substantially surpasses that of the non-treatment group.

This study underscores the importance of nutritional interventions in managing ASD, shedding light on the potential benefits of a GF/CFD coupled with supplements and digestive enzymes. The findings emphasize the need for a holistic treatment approach that includes nutritional therapy in addressing the multifaceted challenges presented by ASD, particularly in individuals with co-occurring GI symptoms. Further research in this domain is encouraged, as it may open new therapeutic avenues and improve the quality of life for individuals affected by this prevalent developmental disorder.

KEY WORDS: Autism Spectrum Disorder, Gastrointestinal Symptoms, Gluten-Free, Casein-Free Diet, Digestive Enzymes, Nutritional Supplements.

1. INTRODUCTION

Autism Spectrum Disorder (ASD) is a multifaceted neurodevelopmental condition characterized by a diverse range of social, communicative, and behavioral challenges. Its diagnosis has witnessed a notable increase in recent years, with the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) estimating a prevalence of 1 in 68 children in the United States (APA, 2013). Subsequent surveillance data reveal that this prevalence continues to surge, with approximately 1 in 54 children diagnosed with ASD (Baio et al., 2018; Maenner et al., 2020). This upward trend in ASD diagnoses has fueled extensive research endeavors to elucidate the condition, including investigations into potential contributing factors (Buie & Furuta, 2015; Wang et al., 2011).

While genetic and environmental influences on ASD have been explored (Bai et al., 2019), there is a growing recognition of the co-occurrence of medical and behavioral conditions in individuals with ASD (Soke et al., 2018; Neumeyer et al., 2019). An area of particular interest is the relationship between gastrointestinal (GI) symptoms and ASD. Several studies have reported a higher prevalence of chronic GI symptoms among individuals with ASD compared to the general population (Molloy and Manning-Courtney, 2003; Ghalichi et al., 2016; Adams et al., 2011).

These GI symptoms encompass a range of issues, including abdominal pain, constipation, diarrhea, and bloating (Adams et al., 2011; Adams & Holloway, 2004). The co-occurrence of GI symptoms in individuals with ASD has led to investigations into potential associations between these symptoms and behavioral problems (Ferguson et al., 2019; Mazefsky et al., 2014). Researchers have explored whether the presence of GI symptoms might contribute to emotional and behavioral challenges commonly observed in individuals with ASD. Furthermore, there is ongoing debate about the efficacy of dietary interventions, such as gluten- and casein-free diets (GF/CFD), in ameliorating both GI symptoms and behavioral issues in this population (Keller et al. 2021; Ghalichi et al., 2016).

In addition to GI symptoms, other medical comorbidities and conditions have garnered attention in the context of ASD. For instance, there is evidence suggesting a higher prevalence of epilepsy in individuals with ASD (Mannion and Leader 2013), as well as a potential association between sensory processing issues and GI symptoms (Khalil et al., 2021).

Several studies suggest that special diets can be beneficial for individuals with ASD. One open-label study found that an 8-week diet that avoided allergic foods resulted in benefits in an open study of 36 children with ASD (Lucarelli et al., 1995). One long-term open-label study of 70 children with ASD who followed GF/CFD for one year or longer, found that 81% of the children improved significantly by the third month, with improvements continuing over the next 12 months. Large improvements were observed in social isolation, eye contact, mutism, learning skills, hyperactivity, stereotypic activity, and panic attacks (Knivsberg et al., 2002). A single-blind study of 10 children with autism found that 8 children benefitted from GF/CFD (Elder et

al., 2006). A 12-week, double-blind, cross-over study of a GFCF diet in 15 children with ASD did not find significant benefits, but parents reported benefits that were not identified by the testing (Whiteley et al., 2010). However, a 12-month, randomized, single-blind, placebo-controlled GF/CFD study involving 54 children with ASD found statistically significant benefits in communication sub-scores (Autism Diagnostic Observation Schedule (ADOS) evaluation) in the GF/CF diet group compared to the control group (MedCalc). The parents (who were not blinded) also reported benefits in social interaction, daily living skills, inattention, and hyperactivity (Zickgraf and Mayes, 2019).

2. THE AIM OF THE STUDY

The research was focused on the effect of innovative dietary approaches application on autistic individuals' symptoms. More explicatively to find out whether GF/CFD intervention combined with supplements and digestive enzymes intake reduces and mitigates the severity of behavior and gastro-intestinal symptoms.

This study was not designed to look at the effect of individual treatments (most of them have already been investigated individually); rather the goal was to investigate the effect of a combination of treatments which provide comprehensive, synergistic nutritional support.

In light of this multifaceted interplay between medical conditions and ASD, our endeavor is to contribute to a deeper understanding of the intricate nature of ASD and offer insights that may inform clinical practice, interventions, and healthcare strategies for individuals with this condition.

3. MATERIAL AND METHODS

105 participants of ASD Group treated in the "Olive Medical and Laboratory Hospital" in Pristine, Kosovo were consulted and examined by the physician and while performing the food intolerance test, to each of them was explained and clarified in a simplified way the theoretical connection brain-gut axis. They were invited and asked if they wanted to be part of our study.

These subjects were randomly divided into two groups. Among these 105 autistic subjects only 39 accepted and agreed to be part of the clinical trial as the so-called "nutritional treated group", to undergo strict diet and supplements intake, 42 subjects remained as "the non-treated group" and 24 autistic subjects refused totally to be part of this trial, to undergo controls, medical examinations, routines etc.

Parents and guardians of two groups completed questionnaires about their child's behavior and GI symptoms (Table1). The form was translated in Albanian and parents were clarified before reporting the symptoms severity.

Table 1. The behavior modular adapted and translated in Albanian

7-point scale				
3-much better				
2-better				
1-slightly better				
0-same				
-1-slightly worse				
-2-worse				
-3-much worse				
	Date before	Score	Date after	Score
1. language				
2. cognition/thinking				
3. play skills				
4. sociability				
5. eye contact				
6. tantruming				
7. stools/gut issues				
8. sleep				
9. hyperactivity				
10. total score				

39 ASD participants, with their family support started in the Treatment group, three dropped out, two were disqualified, and thirty-four completed the study. The ASD family members, unlike the rest of the group they were positive, optimistic and willing to undergo checks and changes. But despite their will and desire, two of them followed the gluten-free and lactose-free diet, but their parents failed to obtain the intake of oral supplements. This made their journey worthless and not counted as a subject that underwent the treatment, thus they were excluded from the trial.

Three other parents with autistic participant (boys) resigned after four months after noticing any change or benefit in favor of physical, emotional or medical development. So, effectively in the study remained 34 ASD participants who were evaluated and investigated for the effects of nutritional diet.

42 ASD members started in **non-treatment group** with no diet restriction, six dropped out resigning at different times, so 36 completed the study without making any major changes in their baseline treatments.

Participants in the treated group followed a GF/CFD and received digestive enzymes and nutritional supplements according to a prescribed protocol.

The study used Likert scale forms to assess GI symptoms and behavioral measures. The Parent Global Impressions - Revised (PGI-R) was used to assess changes in symptoms over time.

Likert scale forms to assess GI symptom

The GI symptoms assessed, based on a factor analysis, the 15 GSRS items break down into the following five scales:

1. Abdominal pain (abdominal pain, hunger pains and nausea).
2. Reflux syndrome (heartburn and acid regurgitation).
3. Diarrhea syndrome (diarrhea, loose stools and urgent need for defecation).
4. Indigestion syndrome (borborygmus, abdominal distension, eructation and increased flatus).
5. Constipation syndrome (constipation, hard stools and feeling of incomplete evacuation).

Frequency of the symptoms was rated on a 7-point Likert scale: No discomfort at all -1-point, Minor discomfort – 2 points, Mild discomfort -3 points, Moderate discomfort- 4 points, moderately severe discomfort – 5 points, Severe discomfort – 6 points, very severe discomfort- 7 points.

The Parent Global Impression (PGI-R)

It was a simple list of 8 symptoms, including: Expressive Language, Receptive Language, General Behavior, Gastrointestinal Symptoms, Sleep, Sociability, Eye Contact, and Overall.

The symptoms were rated on a scale of 1-7, where 1 = much worse, 2 = worse, 3 = slightly worse, 4 = no change, 5 =slightly better, 6 = better, 7 = much better. The PGI revised replaces the “General Behavior” category in the PGI with the more specific categories of Hyperactivity, Tantrumming, Cognition, and Play. Also, an Average Change score is computed, based on the average of the individual scores. Finally, the scale is changed from a range of 1-7 to a range of -3 to +3; i.e., -3 = much worse, 0 = no change, and +3 = much better

3.1. Protocol for ASD Treatment Group

3.1.1. Monitoring: Initial evaluation of autism severity and overall functioning level. Physical examination by the study physician to verify that the participant is in sufficient good health to participate in the study.

Following collection of baseline data, participants were monitored throughout the study by telephone and/or SMS for individualized dosing titration and for potential adverse effects. This was done by nutritionist, the nurse that helped with the study supervision and the physician. During initial dosing individualization, monitoring was done weekly, and then bi-weekly (or more often if needed). Initial blood draw and first-morning urine collection were performed before the nutritional and supplemental treatment started.

3.1.2. Dosage: was adjusted based on baseline measured body weight up to a maximum of 45 kg (see Table 12). Based on prior studies dosage levels of nutrients in the supplement were chosen to be significantly higher than RDA levels, but either at or below the Tolerable Upper Limit. The supplement was administered by the parents. Compliance was also monitored.

The study dose was gradually increased during the first 3-4 weeks of the study:

Days 1-6: 1/6 of full dose

Days 7-13: 2/6 of full dose

Days 14-20: 3/6 of full dose
Days 21-27: 4/6 of full dose
Days 28-03: 5/6 of full dose
Days 04 of the other month and later: full dose.

The dosage was individually titrated in cases where parents reported possible adverse effects, with the dosage being lowered temporarily in some cases. By the end of the study, most participants were at the full dose. Thus, for most children the full dosage was well-tolerated, but for approximately 10% a slightly lower dosage was used to reduce or eliminate side-effects.

After 3 months: the first gastro-intestinal assessment was performed, based on Gastrointestinal Symptom Rating Scale (GSRS) questionnaire.

After 6 months: Final assessment of autism severity using parent Global Impression Score and second reevaluating gastro intestinal symptoms and overall functioning status. Final blood draw and urine collection.

3.1.3. Protocol for ASD Non-Treated Group

Initial evaluation of autism severity and overall functioning level. Physical examination by study physician to verify that participant is in sufficient good health to participate in the study. Initial blood draw and first-morning urine collection. Verification of no changes in treatment during last 6 months. Final assessment of autism severity using parent Global Impression Score and overall functioning status.

Final blood draw and urine collection.

3.2. Statistical analysis / Measures

3.2.1. t-test

To determine whether there was difference between groups t –test was performed. It was based on two independent samples. To make this statement, the mean value of the two samples was compared. If the difference in means was large enough, it was assumed that the two groups differ significantly. A two-sided $p < 0.05$ was considered as statistically significant. Pearson test was used to determine the correlation between two groups.

Food intolerance test results, group characteristics and the breakdown by gender and age were processed, obtained and analyzed with Pivot Tables from Microsoft Excel Worksheet.

The percentage was calculated by percentage calculator and the p/value was calculated from Data Analysis from Microsoft Excel Worksheet.

Charts, diagrams and tables were elaborated also from Microsoft Excel Worksheet. Table data, disaggregated by gender and age were processed by Pivot Tables in from Microsoft Excel Worksheet. Behavioral and gastro-intestinal test answers were converted from words to numerical values via “Find and Replace” from Microsoft Excel Worksheet.

The paired t-test was used to calculate the gastro-intestinal p-value symptoms, as there was a difference between two variables for the same subject.

The unpaired t-test was used to compare the behavioral averages/means of two independent or unrelated groups such as “treatment group” and “non treatment group” and to determine if there

is a significant difference between the two of them.

variable tallying the number of symptoms reported for each child with ASD: constipation, diarrhea, abdominal discomfort, gastro-esophageal reflux, vomiting, excessive gas, and bloating.

Data were analyzed using paired t-tests to evaluate changes in GI symptoms and behavioral measures over 3 and 6 months. The obtained results are presented in tabular and graphical formats.

4. RESULTS AND DISCUSSION

Our study is the clinical trial by taking it to another dimension, and giving children the opportunity to benefit from the healthy GF/CFD combined with supplements and digestive enzymes. The characteristics of the study participants are listed in Table 2.

Table 2. Participant characteristics

	ASD–Treated group		ASD–Non–Treated group	
Total Participants	n=34		n=36	
Male	19		25	
Female	15		11	
Age (Years)	1-34 (13.9)		2-37 (16.7)	
Children (ages 1–12)	n=20	59%	n=19	53%
Teens (ages 13–20)	n=1	3%	n=2	6%
Adults (ages 20+)	n=13	38%	n=15	41%

The ASD treated group and ASD non-treated group, have similar age distributions (mostly children, some teens, and a few adults), and similar gender distributions (mostly male).

The group of 36 members that refused totally to be part of the clinical trial, were of considerable age with a range from 38-75 years, with an average age of 48.25 years. As mentioned above, this was also the unmotivated category and with numerous ineffective efforts by family members throughout their life to improve the quality of living. It seemed that participants of this age had given up and accepted the fact that nothing could change their status.

4.1. Nutritional interventions in ASD

Effects of dietary interventions in ASD have recently begun to emerge. It is important to understand what physiological effects dietary interventions may have, because individuals with ASD already exhibit difficult and picky eating (Horvath and Perman, 2002; Cermak et al., 2010). So, it is most important to investigate diets, because they could also aggravate the imbalances in gut microbiota composition and the GIT problems.

Buie et al., indicated that 36 parents (70 %) notified that their child had previously taken at least 1 supplement and 83% of parents reported thinking supplements were safe, the other part indicated that they could use the supplements only under supervision of a health care

professional. Our results, the effect after 3 and 6 months of implementing GF/CF diet on gastrointestinal symptoms in ASD subjects is presented on Table 3.

Table 3. The average effect of GF/CF diet on GIT symptoms, after 3-months and 6-months

Variables	Gluten free/ Casein Free diet (n=34)				
	Baseline	After 3 months	P-value	After 6 months	P-value
Abdominal discomfort	3.23	2.61	0.003	2.02	0.001
Bloating	3.9	2.9	0.0001	2.2	0.0001
Diarrhea	3	2.8	0.3	2.3	0.02
Nausea	2.73	2.64	0.29	2.61	0.4
Constipation	3.2	2.8	0.05	2.4	0.02
Reflux syndrome	2.85	2.5	0.09	2.7	0.2
Hunger pain	3	3	0.41	2.4	0.005
Urgent need for defecation	3.2	2.7	0.005	2.5	0.07
Burping	3.2	3	0.3	2.4	0.003
Heartburn	3	2.7	0.1	2.7	0.39
Hard stool	3.85	2.97	0.001	2.2	0.0001
Rumbling	3.7	2.82	0.0002	2.17	0.0001
Flatus	3.5	2.7	0.0002	2	0.0001
Loose stool	2.9	2.5	0.11	2.6	0.26
Incomplete emptying	2.8	2.7	0.2	2.6	0.4

As we see from the table there are several significant changes related with ameliorative gastrointestinal symptoms in different subclasses of symptoms. It seems that nutritional intervention, eliminating casein and gluten as well as taking supplements according to the protocol has affected some aspects of the disease, resulting in reducing the severity of some symptoms, such as: abdominal discomfort, bloating, diarrhea, constipation, hunger pain, burping, hard stool, rumbling and flatus.

Before the dietary treatment, 10 (3.4%) ASD members responded that have had very severe symptoms of gastro-intestinal aspect, related mainly with indigestion such as: flatus, burping, rumbling and bloated with a score of seven points for each question.

Regarding frequency, we can say that 106 responders regarding all subclasses of symptoms, referred to have no discomfort at all. So only 36% reported to have not any gastro-intestinal symptoms related. The remaining part referred problems of various levels, from mild - 82 subjects (27%) to severe with 109 times as frequency.

After 3 months, the first step was the gastro-intestinal evaluating process. The 90th day, the parents were asked to fill out online (for convenience, being the Covid situation) carefully the Likert scale form for gastrointestinal symptoms. This was also the first step to evaluate the route and the effect of GFCF diet and supplements.

Although it was a short time, there were significant and positive changes regarding the improvement of digestive tract symptoms. Improvements were related to abdominal discomfort ($p=0.003$), bloating ($p=0.0001$), constipation ($p=0.05$) hard stool ($p=0.001$), rumbling ($p=0.0002$) and flatus ($p=0.0002$).

In ASD members symptom relief was observed, but we can say that in some of them there was a slight aggravation of symptoms, seen from the increasing scoring system.

After 6 months substantial and significant changes were observed. We calculate the p-value of samples, before and after three and six months, using paired t-test as there was a connection in each of variables and samples, so practically it was the same person who was statistically investigated.

It is important to emphasize two things: first the significance achieved after three months, was managed to remain positive even after six months. So, abdominal discomfort ($p=0.003$), rumbling ($p=0.0002$), hard stool ($p=0.001$) and flatus ($p=0.0002$) deepened their significance, making the p-value lower than that of three months earlier.

Secondly, in the list of symptoms that improved after 180 days and that three months ago had no significant results was diarrhea ($p=0.02$), hunger pain ($p=0.005$) and burping ($p=0.003$). The results are presented at Figure 1.

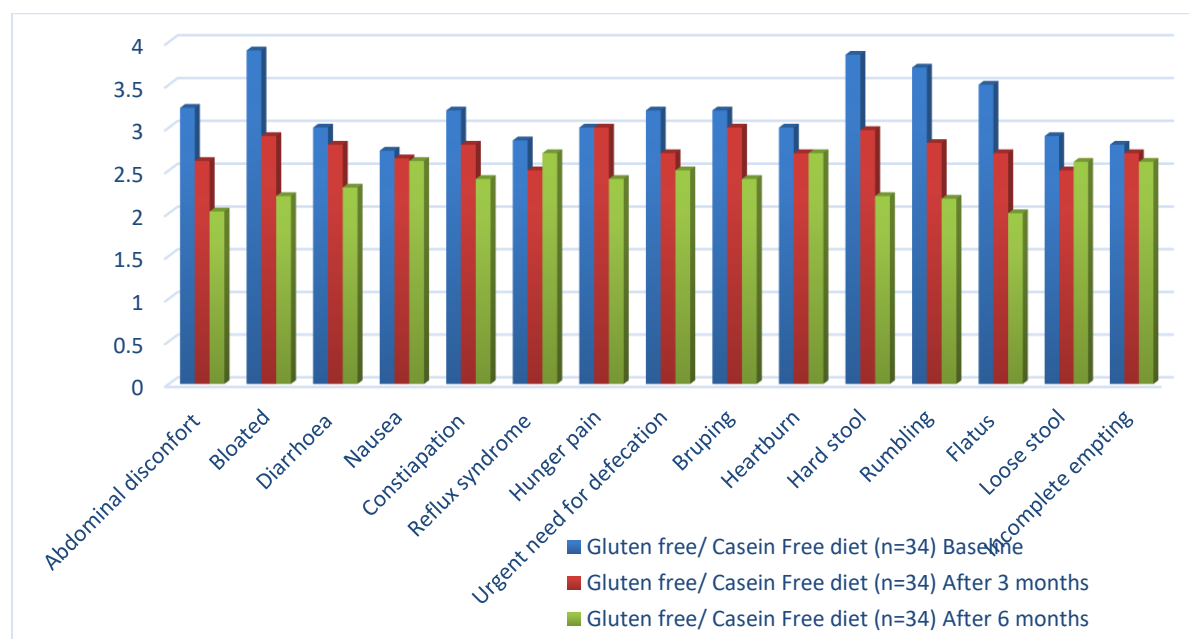


Figure 1. Summary of gastro-intestinal symptoms performance for the course of the study (baseline, after 3 months and after 6 months)

The symptom that worsened was heartburn and nausea $p=0.29$ after three months and $p=0.4$, which however is related to the idea of taking supplements three times per day in some members, and may have caused the feeling of vomiting.

The symptoms that were significantly diminished were abdominal discomfort, constipation,

hunger pain, burping, rumbling and flatus. Gastrointestinal disturbances are commonly encountered comorbidities that are thought to be not only another symptom of ASD but to also play an active role in modulating the expression of social and behavioral symptoms (Keller et al., 2021).

4.2. Effect of GF-CF diet and supplement on behavioral symptoms

One of hypotheses was "Will the treatment group improve more than the non-treatment group on one or more of the measures of autism severity?" The PGI-2 assesses change in symptoms (from beginning to end of study), using a scale ranging from -3 (much worse) to zero (no change) to 1 (slightly better), 2 (better), 3 (much better).

The results for the assessment tools are shown in Table 4 and presented at Figure 2. For the PGI-R Average Change, the Gluten free/Casein free group had a significant improvement compared to ASD non-treated group (5.0 +/- 4.1 vs. 3.7 +/- 2.52, $p = 0.0001$).

Table 4. The effect of GF/CF diet on behavioral measures of treated and non-treated group

Variables	Treated Group (n=34)	Non-Treated Group (n=36)	p-value (two-tail)
Language	0.85	-0.03	<i>n.s</i> 0.006
Cognition/thinkig	0.47	-0.08	<i>n.s</i> (0.08)
Play skills	0.67	-0.33	0.002
Sociability	0.68	-1.19	0.003
Eye contact	0.59	-0.3	0.004
Tantruming	0.14	-0.56	<i>n.s</i> (0.03)
Stools/gut issues	0.35	-0.53	0.004
Sleep	0.82	-0.03	0.003
Hyperactivity	0.47	0.47	<i>n.s</i> (0.01)
Total Score	171	-84	0.0001

There are a total of 9 comparisons of behavior stereotype, such as: language, cognition/thinking, play skills, sociability, eye contact, tantruming, stools/gut issues, sleep and hyperactivity. As the table indicates, significant improvement is seen in play skills ($p\text{-value} = 0.002$), followed by a significant and noticeable enhancement in the sociability field ($p\text{-value} = 0.003$), eye contact and stools/gut issues both with a significant of $p\text{-value} = 0.004$ and significant sleep improvement ($p\text{-value} = 0.003$). There are possible trends towards improvement in the areas of Expressive Language ($p = 0.006$).

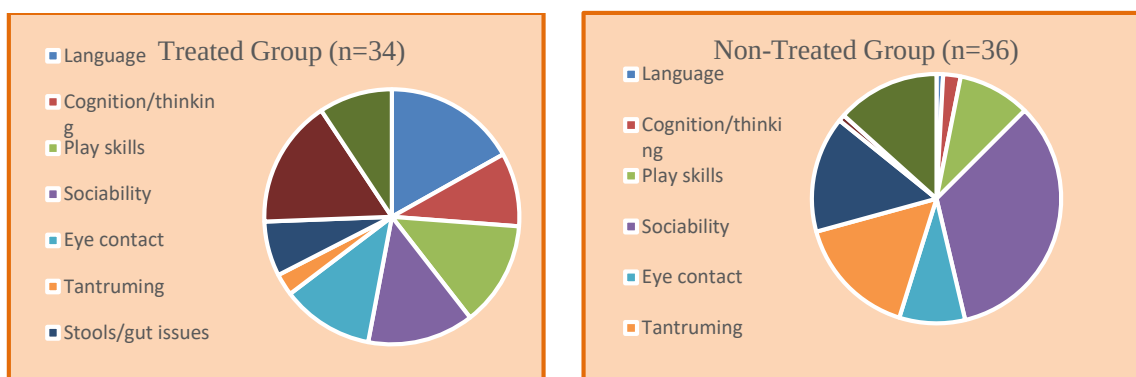


Figure 2. Performance of the two study groups in relation to behavior stereotype

Table 4.1. The difference between the observed means in two independent samples

Difference	-7.570
Standard error	0.952
95% CI	-9.4688 to -5.6712
t-statistic	-7.956
DF	68
Significance level	P < 0.0001

The other areas of the PGI-R yielded non-significant differences between the treated and non-treated group, but the treated group consistently scored higher in those other areas, suggesting that larger studies may be needed to investigate possible differences in those other areas.

The total score of treated Group (n=34) was 171, mean = 5.0 and the Standard deviation =4.1. The non-treated group had a -2.52 mean and the standard deviation 3.79. The difference between the observed means in two independent samples was calculated. A significant p value and 95% Confidence Interval (CI) of the difference was reported. So, when the P-value is less than 0.05 ($P < 0.05$), the conclusion is that the two means are significantly different. P-values are always two-sided (or two-tailed) as the samples are independent from each other.

The results of the Parental Global Impressions Revised are presented on Figure 3.

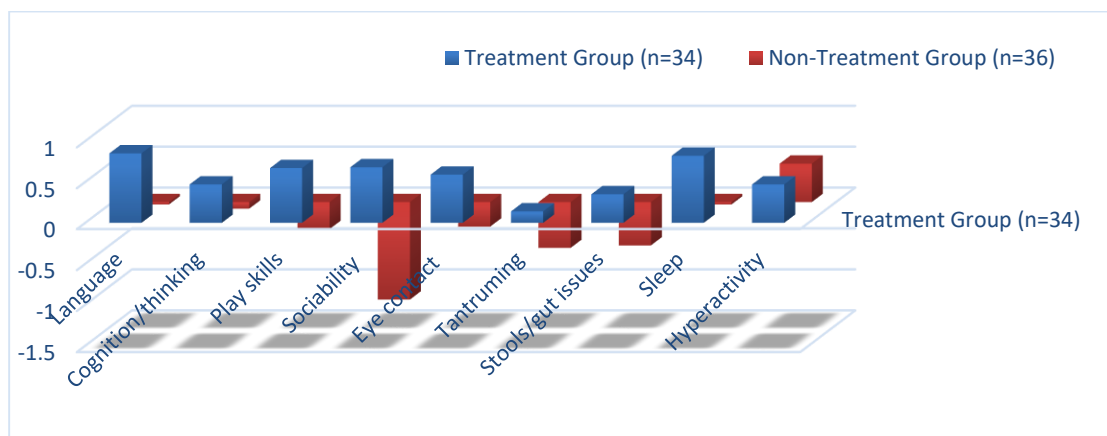


Figure 3. Results for the Parent Global Impressions - Revised. The y-axis is from -3 (much worse) to 0 (no change) to +1 (slightly better) to +2 (better) to +3 (much better)

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by challenges in social communication, restricted interests, and repetitive behaviors (American Psychiatric Association 2013) that currently affects over 2% of 8-year-olds in the US (1 in 44) (Baio et al., 2018; Maenner et al., 2020). Autism often co-occurs with other conditions such as psychiatric disorders (Bai et al., 2019; Croen et al., 2015), attention deficit hyperactivity disorder (ADHD), intellectual disability (ID), sensory processing, immune dysregulation, and gastrointestinal issues (Soke et al., 2018). Recently, the CDC reported that one-third (34%) of autistic children have intellectual disability (ASD + ID) (Maenner et al., 2020). It is well recognized that individuals with ASD + ID present significant deficits and challenges with adaptive behavior and therefore have different behavior intervention requirements than those individuals with ASD without ID or ID only (Hyman et al., 2020; Matson & Shoemaker 2009).

Previous studies have shown no effect of a GF/CF diet on clinician-reported core symptoms of autism (standardized mean difference (SMD) -0.31 (95% CI. -0.89, 0.27)), reported functional level from parents (mean difference (MD) 0.61 (95% CI -5.92, 7.14)) or behavioral difficulties (MD 0.80 (95% CI -6.56, 10.16)). Conversely, a GF/CF diet may cause gastrointestinal side effects (relative risk (RR) 2.33 (95% CI 0.69, 7.90)(Keller et al. 2021).

In conclusion we can state that a high prevalence of food intolerance was identified in ASD children. The results of the present study suggest the presence of food intolerance to multiple food items in children with ASD suggesting their possible roles in ASD symptoms. Increased food-specific IgG4 antibodies are correlated with the severity of ASD with focus on gastrointestinal symptoms and behavior impact.

In a medical nutrition therapy approach, it is beneficial to examine the pathophysiology, food intake, food intolerances, and nutritional behaviors of the individual with ASD to supplement the energy and nutrient deficiencies with food, to ensure a sufficient and balanced diet and to adopt a therapeutic diet approach to alleviate symptoms.

Our results emphasize the value of GF/CF nutritional diet in ASD subjects. A dietary GF/CF

therapy is insufficient alone to effectively treat autism. Same as our study, other studies demonstrate the need to supplement the nutritional deficiencies of autistic patients with fatty acids omega-3, probiotics, vitamins and minerals. A properly designed elimination diet tailored to the patient's individual may also lead to relief of the autism symptoms and the occurrence of gastrointestinal disorders.

Parents and caregivers should therefore be aware of the benefits of nutritional therapy and need for proper monitoring the treatment and dietary intake of patients with ASD.

Parent reports also suggest improvements in behaviors, sensory processing and GI symptoms.

4.2.1. GIS and Language/Communication

Evidence concerning the relationship between GIS and language and communication is also somewhat inconclusive. Prosperi et al. (2019) found no difference in the verbal ability of children with and without GIS. He compared ASD children with GIS (n = 30) to those without GIS (n = 55).

Neumeyer (2019) conducted a large study with children registered with the 15 Autism Speaks, autism treatment network registry sites. They used clinician-reported diagnoses in two ages groups of children under (n = 2114) and over six years old (n = 1221). This study found that constipation was associated with speech problems in both age groups: (younger children, 8.09%; $p < 0.001$; older children, 6.39%; $p < 0.001$). In addition, speech problems were also associated with feeding problems: (younger children, 9.74%; 1.23; $p < 0.001$; older children, 4.42%;).

This latter study also found that younger, more socially impaired and nonverbal children had increased odds of constipation. However, other previous research found no difference between the verbal ability of children with and without reported pain abdominal pain, or constipation (Molloy & Manning-Courtney 2003), or rates of GI complaints (Ferguson et al. 2019).

4.2.2. GIS and Challenging Behavior

The presence of challenging behaviors is associated with the presence of GIS, especially those with reduced verbal ability (Ghalichi et al. 2016). The number of GIS has also been positively associated with aggression and attention problems (Mannion & Leader 2013; Mazurek et al. 2014) somatic complaints (Niehus & Lord 2006) and affective problems (Mazurek et al. 2014).

Ferguson (2019) found age differences in the association between problem behavior and GIS. They examined the relationships between GIS, problem behaviors, and internalizing symptoms in an older group of children with ASD aged from 6 to 18 and a younger group aged from two to five. They found that children with aggressive behaviors (11.2%) were more likely to experience nausea. In the younger group, aggressive behavior was a significant predictor of nausea ($p < 0.05$), and aggression may be used to communicate GI discomfort (Ferguson et al. 2019). In the older group, children with anxiety were 11% more likely to have constipation and 9% less likely to have stomach aches. Those with more withdrawn behavior (10.9%), were more likely to have stomach aches but 8.7% less likely to experience constipation. Whereas children with somatic complaints were 11.4% more likely to experience nausea and 11.5% more likely to experience

stomach aches.

The recent research findings align with previous research that found GIS were not significant predictors of aggression (Mazurek et al. 2013) and tantrum behaviors were more common in those with GIS (Mannion & Leader 2013) and are predicted by diarrhea (Leader et al. 2021). Previous research also found that nausea predicted conduct, avoidant and worry/depressed behavior, and constipation and abdominal pain predicted conduct behavior (Leader et al. 2021).

4.2.3. GIS and Comorbid Psychopathology

Recent research regarding GIS and comorbid psychopathology has added valuable additional knowledge concerning psychiatric outcomes and particularly concerning anxiety. Leader et al. (2021) documented high levels of comorbid psychopathology in ASD, and parents reported atypical levels of anxiety, depression, and poor mental health, and over a third (37.7%) of children with psychiatric symptoms experienced at least one GIS. Anxiety and somatic complaints are associated with GIS (Neuhaus et al., 2018; Williams et al., 2015). However, there was limited variance in psychiatric outcomes due to GIS and more association in younger children with more GIS (Leader et al. 2021). This recent research concurs and builds upon previous research that GIS are related to anxiety (Mannion & Leader, 2013; Neuhaus et al. 2018).

4.2.4. GIS and Sleep Problems

Several studies examined GIS and sensory issues and they revealed that sensory issues are common in ASD (Khalil et al., 2021). The studies report that sensory issues are exhibited more frequently by children with ASD and adolescents in comparison to children with TD (Khalil et al., 2021) and that their presence was associated with increased rates of feeding problems and GIS (Leader et al., 2021). The highest levels of impairments were found in the "under responsiveness/seek sensation" category (Khalil et al., 2021). This finding somewhat contrasts with previous work that found GI problems associated with sensory over-responsivity (Mannion & Leader, 2013).

Our study hopes to pave the way for new therapeutic goals and interventions, providing hope to individuals afflicted with this prevalent developmental disorder.

This is supported by the increase in non-verbal IQ (nonverbal intelligence), and the substantial 6-month increase in developmental ability in communication, daily living skills, and social skills. Modest improvements were reported in sleep and eye contact.

So, this comprehensive treatment approach is recommended as a promising therapy for children and adults with ASD, with an emphasis on the vitamin/mineral supplement and essential fatty acids as probably being the most helpful.

The nutritional supplements and healthy diet Gluten Free- Casein Free improved nutritional status, and hence presumably increased the brain's ability to function and learn.

The data from this study serves as a useful guide for future dietary interventions CF-GF for children with autism.

5. CONCLUSION

Based on our results obtained in 2 parts of the research, we can conclude the following:

1. The gastrointestinal symptoms that were significantly diminished after the nutritional diet GF/CG were abdominal discomfort, constipation, hunger pain, burping, rumbling and flatus.
2. GF/CFD initiated a significant improvement in behavioral symptoms, especially in play skills, followed by a significant and noticeable enhancement in the sociability field, eye contact and stools/gut issues. There were possible trends towards improvement in the areas of expressive language
3. The nutritional supplements and GF/CFD improved nutritional status, and hence presumably increased the brain's ability to function and learn.
4. There was an increase in non-verbal IQ (nonverbal intelligence), in communication ability, daily living skills, and social skills after period of 3 and 6 months diet implementation.
5. Comprehensive treatment approach is recommended as a promising therapy for children and adults with ASD, with an emphasis on the vitamin/mineral supplement and essential fatty acids as probably being the most helpful.
6. The data from this study serves as a useful guide for future dietary interventions with CF/GFD for children with autism for a longer period. The study hopes to pave the way for new therapeutic goals and interventions.

REFERENCE

1. Adams, J.B., & Holloway, C.E. 2004. Gastrointestinal symptoms are prevalent in children with autism spectrum disorders. *Neurogastroenterol. Motil.*, 16(5), 515-521.
2. Adams, J.B., Holloway, C., & George, F. (2006). Gastrointestinal flora and gastrointestinal status in children with autism—comparisons to typical children and correlation with autism severity. *BMC Gastroenterol.*, 6(1), 34.
3. Adams, J.B., Johansen, L.J., Powell, L.D., Quig, D., & Rubin, R.A. 2011. Gastrointestinal flora and gastrointestinal status in children with autism—comparisons to typical children and correlation with autism severity. *BMC Gastroenterol.*, 11(1), 22.
4. American Psychiatric Association. 2013. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*, 5th ed. American Psychiatric Publishing, Inc.: Arlington, VA, US.
5. Baio, J., L Wiggins, DL Christensen, MJ Maenner, J Daniels, Z Warren, et al. 2018. "Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014." *MMWR Surveill Summ* 67:1–23.
6. Buie, T.; D.B. Campbell; G.J. Fuchs; G.T. Furuta; J. Levy; J. Vandewater; A.H. Whitaker; D. Atkins; M.L. Bauman; A.L. Beaudet; et al. 2010. "Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: A consensus report." *Pediatrics* 125:S1–S18.
7. Buie, T., & Furuta, G.T. 2015. Evaluation of gastrointestinal symptoms in children with autism spectrum disorder and gastrointestinal symptoms. *J. Pediatr. Gastroenterol. Nutr.*, 60(5), 586-587.
8. Cermak, S.A.; C. Curtin; L.G. Bandini. 2010. "Food Selectivity and Sensory Sensitivity in Children with Autism Spectrum Disorders." *J. Am. Diet. Assoc.* 110:238–246.

9. Coury, D.L., Ashwood, P., Fasano, A., Fuchs, G., Geraghty, M., & Kaul, A. 2012. Gastrointestinal conditions in children with autism spectrum disorder: Developing a research agenda. *Pediatrics*, 130(Supplement 2), S160-S168.
10. Elder, J.H.; M Shankar; J Shuster; D Theriaque; S Burns; L Sherrill. 2006. "The gluten-free, casein-free diet in autism: Results of a preliminary double-blind clinical trial." *J. Autism Dev. Disord.* 36:413–420.
11. Erickson, C.A., Stigler, K.A., Corkins, M.R., Posey, D.J., & Fitzgerald, J.F. 2005. Gastrointestinal factors in autistic disorder: A critical review. *J. Autism Dev. Disord.*, 35(6), 713-727.
12. Ghalichi, F.; J Ghaemmaghami; A Malek; A Ostadrahimi. 2016. "Effect of gluten free diet on gastrointestinal and behavioral indices for children with autism spectrum disorders: A randomized clinical trial." *World J. Pediatrics* 12:436–442.
13. Horvath, K.; J.A. Perman. 2002. "Autistic disorder and gastrointestinal disease." *Curr. Opin. Pediatr.* 14:583–587.
14. Horvath, K., Papadimitriou, J.C., Rabsztyrn, A., & Drachenberg, C. 1999. Gastrointestinal abnormalities in children with autistic disorder. *J. Pediatr.*, 135(5), 559-563.
15. Khalil, M.; H.G. Azouz; S.A. Ahmed; H.A. Gad; O.M. Omar. 2021. "Sensory Processing and Gastrointestinal Manifestations in Autism Spectrum Disorders: No Relation to *Clostridium difficile*." *J. Mol. Neurosci.* 71:153–161.
16. Knivsberg, A.M.; K.L. Reichelt; T. Høien; M. Nodland. 2002. "A randomised, controlled study of dietary intervention in autistic syndromes." *Nutr. Neurosci.* 5:251–261.
17. Lucarelli, S.; T. Frediani, A.M. Zingoni, F. Ferruzzi, O. Giardini, F. Quintieri, M. Barbato, P. D'eufemia, E. Cardi. 1995. "Food allergy and infantile autism." *Panminerva Med.* 37:137–141.
18. Maenner, M.J., K.A. Shaw, J. Baio, A. Washington, M. Patrick, et al. 2020. "Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016." *MMWR Surveill Summ* 69:1–12.
19. Mannion, A.; G. Leader. 2013. "An analysis of the predictors of comorbid psychopathology, gastrointestinal symptoms, and epilepsy in children and adolescents with autism spectrum disorder." *Res. Autism Spectr. Disord.* 7:1663–1671.
20. MedCalc. "Statistical software for Windows Desktop." doi: https://www.medcalc.org/calc/comparison_of_means.php.
21. Molloy, C.A.; P. Manning-Courtney. 2003. "Prevalence of chronic gastrointestinal symptoms in children with autism and autistic spectrum disorders." *Autism* 7:165–171.
22. Wang, L.W., Tancredi, D.J., & Thomas, D.W. 2011. The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected members. *J. Dev. Behav. Pediatr.*, 32(5), 351-360.
23. Walker, S.J., Fortunato, J., Gonzalez, L.G., & Krigsman, A. 2013. Identification of unique gene expression profile in children with regressive autism spectrum disorder (ASD) and ileocolitis. *PLoS ONE*, 8(3), e58058.
24. Whiteley P., Haracopos D., Knivsberg A.M. et al. (2010). The ScanBrit randomized, controlled, single-blind study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders. *Nutr. Neurosci.* 13, 87-100.
25. Zickgraf, H.; S.D. Mayes. 2019. "Psychological, health, and demographic correlates of atypical eating behaviors in children with autism." *J. Dev. Phys. Disabil.* 31:399–418.