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CORRELATION BETWEEN CLINICAL MANIFESTATIONS OF GUT DYSBIOSIS AND BEHAVIORAL CHANGES IN CHILDREN WITH AUTISM SPECTRUM DISORDER

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ABSTRACT

Introduction: Autism Spectrum Disorder (ASD) is a neurodevelopmental condition frequently associated with gastrointestinal symptoms, which may influence behavior and quality of life. **Objective:** This study aimed to investigate the presence and association between gastrointestinal symptoms and behavioral manifestations in children with ASD. **Methodology:** This is an observational, descriptive, and quantitative study based on the analysis of questionnaires applied to the caregivers of 21 children under gastroenterological follow-up. **Results:** The results showed a high frequency of constipation (52.4%), abdominal distension (42.9%), and bloating (57.1%). A significant correlation was observed between abdominal pain and the act of pointing to the abdomen ($r = 0.580$; $p = 0.006$), and between nausea/vomiting and irritability ($r = 0.555$; $p = 0.009$), suggesting that physical discomfort may be expressed through nonverbal behaviors. There was also an association between age and irritability ($r = 0.510$; $p = 0.018$), indicating that neurological maturity influences how symptoms are expressed. **Conclusion:** Gastrointestinal manifestations are frequent in children with ASD and are related to behavioral alterations, reinforcing the importance of a multidisciplinary approach and the systematic clinical investigation of these symptoms.

KEYWORDS: Autism Spectrum Disorder; Gastrointestinal symptoms; Behavior; Gut microbiota; Gut-brain axis.

1. INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by impairments in communication, difficulties in social interaction, and the presence of restricted interests and repetitive behaviors. It is a heterogeneous condition that may vary in intensity and often requires continuous support throughout life.[1] In recent years, the prevalence of ASD has increased, with estimates indicating that approximately 1 in 44 children aged eight years had been diagnosed with the disorder in 2018.[2]

Beyond its core symptoms, ASD is associated with various medical comorbidities, among which gastrointestinal disorders stand out due to their high frequency compared to the neurotypical population.[3] In this context, the study of the gut microbiota has gained relevance, particularly due to its bidirectional connection with the central nervous system, known as the gut–brain axis. Alterations in this microbial balance have been linked to both gastrointestinal manifestations and behavioral characteristics of the disorder.[4]

The intestinal microbiota is composed of trillions of microorganisms that play essential roles in digestion, metabolism, and immune regulation. In children with ASD, studies have described significant changes in this ecosystem, including reduced bacterial diversity and decreased abundance of genera such as Bifidobacterium, findings that correlate with greater clinical severity.[5]

These alterations may affect multiple biological systems. Metabolites produced by intestinal bacteria, such as short-chain fatty acids, influence intestinal barrier

integrity, neuronal signaling, and immune response, acting as potential mediators between dysbiosis and ASD symptoms.[6] Moreover, the microbiota participates in the modulation of neurotransmitters such as serotonin and dopamine, reinforcing its relevance at the interface between gut health and brain function.[7]

Given this evidence, there is growing interest in therapeutic strategies aimed at restoring microbiota balance. Dietary interventions, the use of probiotics, and even fecal microbiota transplantation have shown positive effects on improving gastrointestinal and behavioral symptoms in individuals with ASD.[8] Although no specific microbial signature for the disorder has yet been identified, the consistent findings of reduced microbial diversity reinforce the potential of this field as a target for new clinical approaches.

Thus, investigating the relationship between gut microbiota and ASD represents not only an opportunity to better understand the mechanisms involved in the disorder but also a promising avenue for developing interventions that enhance the quality of life of patients and their families.

In light of this scenario, the present study aims to investigate the association between intestinal dysbiosis and the presence of gastrointestinal and neurobehavioral symptoms in children diagnosed with ASD, seeking to understand how microbiota alterations may contribute to the manifestation and intensification of the clinical picture. The goal is to provide evidence that may guide more effective clinical practices and support the development of care strategies that improve the quality of life of affected children and their families.

2. MATERIALS AND METHODS

This is a cross-sectional, observational, and quantitative study aimed at investigating the association between intestinal dysbiosis and the presence of gastrointestinal and neurobehavioral symptoms in children diagnosed with Autism Spectrum Disorder (ASD).

2.1 POPULATION AND SAMPLE

The sample consisted of children aged between 2 and 17 years who were followed at a pediatric gastroenterology clinic in the city of Londrina (Paraná, Brazil). All participants had a diagnosis of Autism Spectrum Disorder (ASD) and presented clinical signs and symptoms of dysbiosis. The selection was carried out by convenience sampling, according to the following criteria

Inclusion Criteria

- Children aged **2 to 7 years** with a diagnosis of Autism Spectrum Disorder (ASD), whose legal guardians signed the **Informed Consent Form (ICF)**
- Children aged **7 to 17 years** with a diagnosis of Autism Spectrum Disorder (ASD), whose legal guardians signed the **Informed Consent Form (ICF)** and who themselves signed the **Assent Form (AF)**, when applicable.

Exclusion Criteria

- Previous diagnosis of genetic conditions associated with behavioral disorders
- Current use of antibiotics
- Previously diagnosed inflammatory bowel disease
- Diagnosis of food allergies or intolerances
- Celiac disease
- Intestinal parasitosis

2.2 DATA COLLECTION PROCEDURES

Data were collected using the **Autism Spectrum Disorder Gastrointestinal and Related Behaviors Inventory.**[10] which was translated into Portuguese by the researchers themselves. The questionnaire was completed exclusively by the children's legal guardians.

The instrument was made available in digital format via **Google Forms** and was sent through the **WhatsApp** application, along with the **Informed Consent Form (ICF)** and the **Assent Form (AF)**, when applicable.

Data collection flow

1. Invitation to participate sent via WhatsApp;
2. Submission of the ICF (and AF, when applicable) in digital format;
3. Acceptance of the terms by the legal guardians;
4. Provision of the Google Forms link containing the ASD-GIRBI questionnaire;
5. Completion of the questionnaire by the legal guardians;
6. Automatic storage of responses in a protected spreadsheet, ensuring confidentiality and data privacy.

2.3 DATA ANALYSIS

Categorical variables (such as the presence or absence of gastrointestinal symptoms) were described using absolute and relative frequencies. Continuous variables (such as age or symptom intensity) were analyzed using measures of central tendency (mean, median) and dispersion (standard deviation).

To assess associations between intestinal dysbiosis (inferred from the symptoms reported in the questionnaire) and neurobehavioral manifestations, statistical correlation tests (Spearman or Pearson, depending on data normality) and association tests (chi-square test for categorical variables) were applied. When appropriate, group comparison tests (Student's t-test or Mann–Whitney test, according to variable distribution) were conducted.

A significance level of $p < 0.05$ was adopted, with associations below this threshold considered statistically significant. The JASP software was used for statistical analyses.

3. RESULTS

A total of 53 patients diagnosed with Autism Spectrum Disorder (ASD) and followed at a pediatric gastroenterology clinic in the city of Londrina were contacted. Of these, 21 responded to the questionnaire and comprised the final sample, with 81% being male and 19% female. It was observed that 71.4% had a previous diagnosis of some gastrointestinal disorder.

Among the reported gastrointestinal symptoms, the most frequent were a sensation of abdominal bloating (57.1%), excessive gas (52.4%), and constipation (52.4%). Abdominal pain and distension/bloating were observed in 42.9% of cases, while diarrhea occurred in 38.1%. Nausea, vomiting, or retching were mentioned by 33.3% of participants, and gastroesophageal reflux was reported by 33.3% (Table 1).

Table 1: Frequency of variables: sex, diagnosis of gastrointestinal disorders, and gastrointestinal symptoms.

Variables	N	%
Sex Female Male	 4 17	 19 81
Diagnosis of gastrointestinal disorder Yes No	 15 6	 71,4 28,6
Nausea, vomiting, or retching Yes No	 7 14	 33.3 66.7
Diarrhea Yes No	 8 13	 38.1 61.9
Abdominal pain Yes No Not sure	 9 10 2	 42.9 47.6 9.5
Gastroesophageal reflux or heartburn Yes No Not sure	 7 13 1	 33.3 61.9 4.8
Abdominal distension or bloating Yes No Not sure	 9 10 2	 42.9 47.6 9.5
Feeling of abdominal fullness Yes No Not sure	 12 7 2	 57.1 33.3 9.5
Excessive gas Yes No Not sure	 11 9 1	 52.4 42.9 4.8
Constipation Yes No Not sure	 11 9 1	 28.6 66.7 4.8
Alternation between constipation and diarrhea Yes No Not sure	 5 15 1	 23.8 71.4 4.8
Incontinence (involuntaru loss of urina or feces) Yes No Not sure	 5 15 1	 23.8 71.4 4.8
Fecal retention/incomplete evacuation Yes No Not sure	 5 14 2	 23.8 66.7 9.5

Regarding the duration of symptom manifestation, 52.4% of the children had gastrointestinal symptoms for one year or longer, and 33.3% for between six and eleven months, indicating a predominantly chronic pattern (Table 2). As for bowel movement frequency, 42.9% evacuated more than once per day, 28.6% once per day, and 23.8% less than once per day. Analysis of the Bristol Stool Scale showed a predominance of types 6 (33.3%), 3 (19.0%), and 2 (14.3%), corresponding to both loose and hard stools, reflecting a mixed pattern of constipation and accelerated bowel transit.

Table 2: Frequency according to the duration of gastrointestinal symptom manifestation.

Duration of gastrointestinal symptoms	N	%
In the past 3 months	1	4.8
3 a 5 months	1	4.8
6 a 11 months	7	33.3
1 year or longes	11	52.4
No symptons reported	1	4.8

Regarding behavioral aspects, 61.9% of the children exhibited irritability, restlessness, aggression, or unexplained screaming. In addition, 38.1% verbally reported abdominal pain, and 23.8% pointed to the abdomen as if experiencing pain. Behaviors such as applying pressure to the abdomen occurred in 23.8%, and impairment in daily activities was reported in 52.4% of the participants, being frequent in 9.5% of cases.

Pearson's correlation analysis revealed statistically significant associations between abdominal pain and pointing to the abdomen ($r = 0.580$; $p = 0.006$), as well as between nausea/vomiting and irritability/restlessness ($r = 0.555$; $p = 0.009$), and between nausea/vomiting and applying pressure to the abdomen ($r = 0.539$; $p = 0.012$). Moreover, a negative correlation was observed between nausea/vomiting and impairment in daily activities ($r = –0.442$; $p = 0.045$).

Table 3: Pearson's correlation between gastrointestinal symptoms and observed behaviors in children with ASD.

Symptoms x behavior	Perason's r	Valor de p
Abdominal pain × Applies pressure to the abdomen, pushing or leaning on furniture	0.396	0.076
Abdominal pain × Points to the stomach/abdomen as if in pain	0.580	0.006
Nausea, vomiting, or retching × Applies pressure to the abdomen, pushing or leaning on furniture	0.539	0.012
Nausea, vomiting, or retching × Irritability, restlessness, aggression, or unexplained screaming	0.555	0.009
Nausea, vomiting, or retching × Impairment in daily activities	−0.442	0.045
Diarrhea × Irritability, restlessness, aggression, or unexplained screaming	0.413	0.062
Constipation × Impairment in daily activities	−0.423	.056
Age x × Irritability, restlessness, aggression, or unexplained screaming	0.510	0.018

Other clinically relevant correlations were identified: pointing to the abdomen and applying abdominal pressure ($r = 0.458$; $p = 0.037$), suggesting compensatory behaviors for visceral pain, and age and irritability/restlessness ($r = 0.510$; $p = 0.018$), indicating that older children may have greater body awareness and express discomfort more intensely.

A trend toward association was also observed between age and the presence of nausea/vomiting ($r = 0.399$; $p = 0.073$), as well as between abdominal bloating and bowel irregularity—particularly diarrhea ($r = −0.415$; $p = 0.061$) and alternation between constipation and diarrhea ($r = −0.418$; $p = 0.060$).

Although these trends did not reach statistical significance, they reinforce the pathophysiological pattern described in ASD, in which distension, gas, and bowel irregularity coexist as manifestations of intestinal dysbiosis and altered motility.

4. DISCUSSION

The findings of this study demonstrate a high frequency of gastrointestinal (GI) symptoms among children with ASD, with constipation, bloating, and abdominal distension being the most prevalent. Although the sample was drawn from a pediatric gastroenterology clinic — which limits generalizability and may overestimate prevalence — the clinical profile identified is consistent with recent evidence indicating that 40% to 70% of children with ASD present recurrent gastrointestinal symptoms.[9]

Constipation, observed in 52.4% of participants, is described as the most common GI disorder in ASD and is associated with food selectivity, low fluid intake, and altered intestinal motility.[11,2] Intestinal dysbiosis, in turn, may impair the production of short-chain fatty acids and increase intestinal permeability, perpetuating a state of low-grade inflammation.[7]

The persistence of symptoms for more than six months, observed in 85.7% of the sample, reinforces the chronic nature of these alterations. This chronicity suggests that microbial imbalance may affect the immune system by promoting continuous release of inflammatory cytokines (IL-6, TNF-α), which can interfere with both intestinal motility and central neurotransmitter regulation.[13]

The correlation analyses deepen this understanding. The association between abdominal pain and pointing to the abdomen ($r = 0.580$; $p = 0.006$) and between pointing and applying abdominal pressure ($r = 0.458$; $p = 0.037$) indicates that nonverbal behaviors can serve as consistent markers of gastrointestinal discomfort. This finding supports observations by Holingue et al. (2022), who reported that pain symptoms in children with ASD frequently manifest atypically.

The correlation between nausea/vomiting and irritability/restlessness ($r = 0.555$; $p = 0.009$) confirms that physical discomfort can precipitate emotional changes, while the negative correlation between nausea/vomiting and performance in daily activities ($r = –0.442$; $p = 0.045$) underscores the functional impact of GI symptoms on daily routines, school performance, and social interaction. These findings support the gut–brain axis hypothesis, a bidirectional system in which alterations in the microbiota and intestinal permeability modulate neurotransmission (serotonin, GABA, dopamine) and influence behavior and mood (CHERNIKOVA et al., 2021; GONÇALVES et al., 2024).

Another relevant finding was the association between age and irritability/restlessness ($r = 0.510$; $p = 0.018$), suggesting that neurological maturity and increased body awareness may intensify behavioral expression of physical discomfort. Similarly, the trend between age and nausea/vomiting ($r = 0.399$; $p = 0.073$) indicates that older children are more capable of recognizing and reporting gastrointestinal symptoms, whereas younger children rely predominantly on behavioral cues.

Furthermore, the trends observed between bloating and diarrhea ($r = –0.415$; $p = 0.061$) and between bloating and alternating bowel habits ($r = –0.418$; $p = 0.060$) reinforce the clinical pattern of bowel irregularity associated with dysbiosis — a phenomenon widely described in recent studies.[11,12] Although not statistically significant, these trends are clinically relevant, especially considering the small sample size.

From a clinical standpoint, these findings emphasize the importance of systematically evaluating GI symptoms in patients with ASD, even when pain is not verbally expressed. Behaviors such as abdominal pressure, unexplained irritability, or social withdrawal may indicate underlying physical discomfort. Reviews published in Brazilian journals.[12] further expand this understanding by emphasizing that food selectivity, characteristic of ASD, contributes to nutritional deficiencies and reinforces the cycle of dysbiosis and intestinal inflammation, with repercussions for cognitive and emotional symptoms. These reviews also highlight the relevance of multidisciplinary approaches and individualized dietary plans, including the judicious use of probiotics and supplementation with essential fatty acids. Integrated interventions — encompassing nutritional monitoring, probiotic therapy, and behavioral strategies — have shown benefits in improving both gastrointestinal symptoms and reducing irritability and agitation.[8]

Finally, the results of this study reinforce that gastrointestinal symptoms in ASD should not be viewed as peripheral manifestations, but rather as part of a shared pathophysiological and behavioral axis, in which intestinal and emotional alterations mutually reinforce one another. Recognizing and addressing this interaction may represent a crucial step toward improving the well-being and quality of life of children with ASD and their families.

5. CONCLUSION

The present study identified a consistent association between gastrointestinal symptoms and behavioral manifestations in children with Autism Spectrum Disorder (ASD), particularly between abdominal pain and nonverbal pain behaviors such as pointing to or pressing the abdomen, and between nausea/vomiting and irritability. These findings reinforce the notion that digestive alterations may underlie behavioral expressions often interpreted solely as intrinsic features of ASD.

Constipation, abdominal distension, and irregular bowel habits emerged as the most prevalent symptoms, forming a clinical profile consistent with intestinal dysbiosis and gut–brain axis dysfunction. This axis, which connects the microbiota, immune system, and neurotransmitters, may explain the coexistence of gastrointestinal discomfort and emotional alterations, supporting the need for a broader and integrative diagnostic approach.

The data also suggest that age influences the way discomfort is expressed, with older children demonstrating greater body awareness and verbalization of symptoms, whereas younger ones tend to manifest pain through atypical behaviors. This observation has direct implications for clinical practice, emphasizing the importance of careful observation and interpretation of nonverbal signs during the assessment of patients with ASD.

Thus, the study highlights that the evaluation of gastrointestinal symptoms should be an integral component of ASD management, not only to improve physical comfort but also for its potential to reduce maladaptive behaviors and enhance quality of life. Future studies with larger samples and longitudinal follow-up are warranted to clarify the causal mechanisms

of this relationship and to inform more effective therapeutic strategies, including nutritional interventions and microbiota modulation.

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CONFLICT OF INTERESTS
This study has no conflict of interest.

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