PRELIMINARY DATA ON THE LINK BETWEEN THE INTESTINAL MICROBIOME AND AUTISM SPECTRUM DISORDERS

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PRELIMINARY DATA ON THE LINK BETWEEN THE INTESTINAL MICROBIOME AND AUTISM SPECTRUM DISORDERS (Abstract): Today autism is an extremely serious problem. Although the number of the children born with this pervasive developmental disorder has increased, the causes of its occurrence have not yet been identified. **Aim:** We want to demonstrate that the severity of gastrointestinal disorders would be related to the worsening of symptoms in people diagnosed with autism spectrum disorders (ASD). Analyzing the intestinal microbiome is particularly important for people with autism because an imbalance in the intestinal microbiome affects their good physical and mental development. **Materials and methods:** In our study, we highlighted the importance of a suitable diet for a better development of children with autism. Children with ASD are at a significantly increased risk of chronic gastrointestinal problems from an early age. The underlying mechanism to explain how gastrointestinal dysfunction can affect autism spectrum disorder behaviors has led to the idea that the microbiome-gut-brain axis contributes to behavioral and cognitive disorders. **Results:** These children have a high prevalence of intestinal transit disorders such as constipation, a fact that influences the severity of symptoms in ASD. The results of our research indicate an increased prevalence of gastrointestinal symptoms among children with ASD compared to the control group. **Conclusions:** Intestinal dysbiosis can influence the severity of symptoms in autism. An understanding of the mechanisms by which the microbiome-gut-brain axis acts will lead to the use of therapeutic strategies. **Keywords:** INTESTINAL MICROBIOME, DYSBIOSIS, AUTISM SPECTRUM DISORDERS.

The specificity of children with autism spectrum disorders (ASD) is the way in which they manifest behaviorally, the withdrawal from social life or even from reality, but also the fact that autism affects such young and innocent children, depriving them of the opportunity to enjoy life. The development of children with autism, later of adults, is affected by this isolation from the environment in which they live. Although these people live in society and have families, they fail to integrate properly. The destructive character of autism is mainly the detachment from the social environment. Fecal analysis can provide clues about intestinal microbial metabolism. However, most studies focus on metabolites in urine and blood. Only a few studies have examined the gut microbiota and the metabolic products of the microbiota and attempted to correlate them with ASD. Wang *et al*. have observed increased concentrations of short-chain fatty acids (SCFA) and ammonia in the feces of children with ASD (1). The gut microbiota plays an essential role in human health and in various disorders such as ASD. A growing body of preclinical research suggests that gut dysbiosis may influence brain function and social behavior, but little is known about the mechanisms underlying these interrelationships and how they may influence pathogenesis or severity in ASD (2). In their research, Angelis *et al.* reported a decrease in total SCFA in children with ASD (3). They also observed altered levels of the neurotransmitter's glutamate and GABA in feces samples. Glutamate was highest among children with autism and GABA was lowest among children with pervasive developmental disorder not otherwise specified (PDD-NOS). In addition, phenolics, including p-cresol, were higher in fecal samples from children with autism and PDD-NOS, but phenolic and p-cresol levels were similar between children with ASD and children from control group (3). ASD patients with gastrointestinal symptoms have changes in microbial composition that are likely related to digestive enzyme deficiencies, carbohydrate malabsorption, selective eating, bacterial toxins, serotonin metabolism, and inflammation (4). The high prevalence of gastrointestinal disorders among ASD patients has led researchers to consider the gut microbiota as a possible factor in the pathogenesis of ASD. Thus, many studies have linked gut dysbiosis, which is frequently observed in patients with autism spectrum disorders, to the modulation of brain function and social behavior, but little is known about this relationship and contribution to the etiology of ASD (5, 6, 7). Hill *et al*. noted that people with ASD prefer energy-dense, nutrient-deficient foods and reject fruits, vegetables, and whole grains (8). The introduction of a new food or physical activity can often cause disruptive behavior that ultimately leads parents to give in to their children's demands (9). Therefore, new foods should be introduced to these children gradually to ensure familiarity with the taste and texture of the food (10).

Parents should teach babies good eating habits from the time the babies start to eat solid food. Autism spectrum disorders can have various social implications, both for people diagnosed with this developmental disorder, as well as for their families and society in general. The literature reports that parents of these children experience increased psychological distresses, including depression, anxiety, interpersonal sensitivity, hostility, schizoid traits, paranoia, and schizophrenia (11). Stressors relate to both

family and social life and include the emotional aspects of a disabled child, understanding the child's needs, various aspects of primary care, medical and educational services, and financial difficulties (12,13).

Due to the high incidence of ASD, as well as the large number of medical services that affected children need, the impact of ASD on the family and the state budget becomes increasingly important. Financial resources allow services to be planned, resources to be allocated and policies to be modified accordingly (14). Medical costs for children with ASD average 3 to 9 times higher than for other children (15).

Therefore, further elucidation of microbiota composition will provide a novel and promising therapeutic approach to control or prevent symptomatology exacerbation in autism. There is growing evidence that the microbiota-gut-brain axis can influence gastrointestinal health and modify those behaviors associated with ASD. Gut microbiota and the metabolic products of microbial populations may be linked not only to gastrointestinal problems but also to behavioral symptoms in autism. In particular, we focus on how gut dysbiosis may affect intestinal epithelial permeability, immune function and microbial metabolites in individuals with autism. To better understand the structure of the gut microbiota in children with autism spectrum disorders of different ages, as well as the relationship between the gut microbiota and fecal metabolites, we need to assess the gut microbial population.

The purpose of this research is to identify if there are significant differences between the intestinal microbiome analysis in the case of children with ASD compared to the control group consisting of neurotypical children.

MATERIALS AND METHODS

We aimed to identify if there is a causal link between intestinal transit disorders such as chronic constipation, the increased level of psychomotor agitation, frequent colds and the presence of an imbalance in the intestinal microbiome bioindicators. We also wanted to observe if there are differences between the values of bioindicators from the intestinal microbiome obtained by the participants from the two research groups.

Objectives

O1. Carrying out the analysis of the microbiome from fecal matter in subjects with ASD and those in the control group.

O2. Establishing how the intestinal imbalance influences the severity of symptoms.

O3. Identification the sort of enterotype of the research participants.

Hypotheses

HS1.It is assumed that there are statistically significant differences between the results of bioindicators from the intestinal microbiome in children with ASD and in neurotypical children.

Hs2. It is assumed that the presence of transit disorders such as constipation can be a cause of imbalances in the intestinal microbiome.

Hs3. It is assumed that the disturbance of the profile of intestinal bacteria can influence the behavioral manifestations.

Ethics approval. In order to participate in this research, the parents of children with ASD and those in the control group have signed a consent agreement.

Study participants. A number of 16 children have participated in this study, 8 of whom have been diagnosed with ASD,

have an increased psychomotor agitation, frequently catch colds due to low immunity and have intestinal transit disorders such as constipation, and 8 are children without disabilities. The children from the first group have been diagnosed with infantile autism and severe developmental delay before the age of 3 and have started psychological and speech therapy immediately after diagnosis at the Psychological Centers in the city of Constanţa.

The ages of the participants in this study are between 3 and 17 years. The groups are similar in terms of age and gender and all live in Constanţa.

Inclusion and exclusion criteria

Group 1. ***Inclusion criteria:*** diagnosis of ASD; informed consent signed by the parents; constipation – defined as the condition of eliminating bowel contents with a frequency less than 3 stools per week (16); increased psychomotor agitation; frequent colds due to low immunity. ***Exclusion criteria***: absence of autism diagnosis; lack of transit disorders; absence of consent agreement.

Group 2. ***Inclusion criteria:*** children without disabilities; informed consent signed by the parents. ***Exclusion criteria:*** presence of ASD diagnosis; absence of consent agreement.

Methods. The participants from both groups have been analyzed for the microbiome from fecal matter, analysis carried out at the *Bioclinica laboratories*.

All the data obtained from the analysis of the bioindicators of the intestinal microbiome were statistically analyzed with the *SPSS version 24 program*. The statistical results will be presented in the tables of the paper.

RESULTS

Enterotypes distribution

In group 1, four participants (50%) had enterotype 1, two participants (25%) had undetermined enterotype, one participant (13%) had enterotype 2 and one participant (13%)had enterotype 3 (fig. 1). In group 2, all eight subjects (100%) had enterotype 1 (fig. 2).

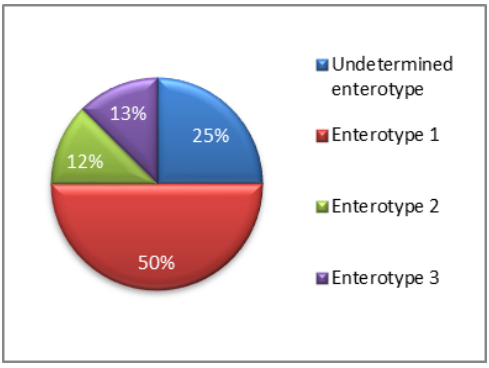

Fig. 1. Distribution of enterotypes group 1

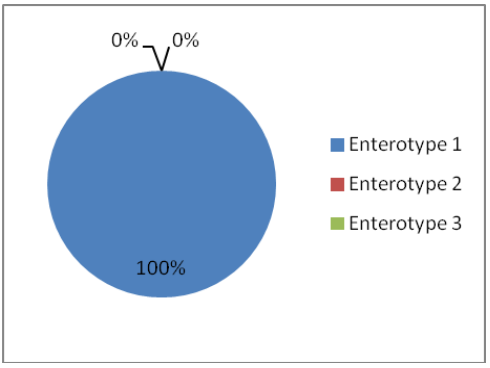

Fig. 2. Distribution of enterotypes group 2

Bioindicators analysis

Fecal pH

In group 1, three participants (38%) had high values, and five participants (62%) presented normal values (fig. 3). None of the participants had low values. In group 2, one participant (13%) had high value, seven participants (87%) had normal values. None of

the subjects presented low values (fig. 4).

Biodiversity (Shannon index)

In group 1, five participants (62%) had low values, three participants (38%) had normal values, while none of the participants presented high values (fig. 5). In group 2, seven participants (87%) had normal values, one participant (13%) had high values, while none of the subjects presented low values (fig. 6).

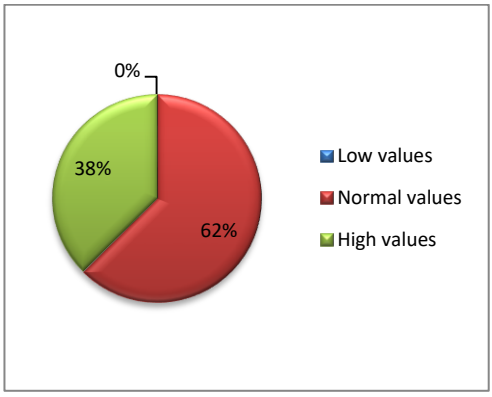

Fig. 3. Fecal pH – group 1

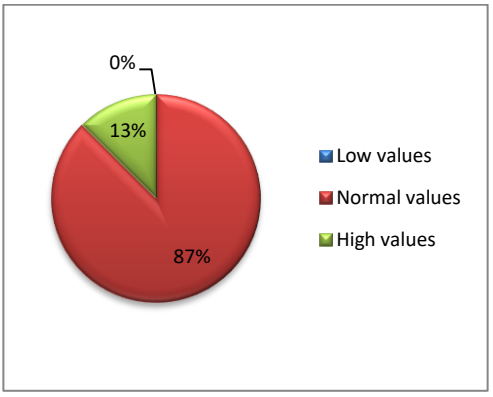

Fig. 4. Fecal pH – group 2

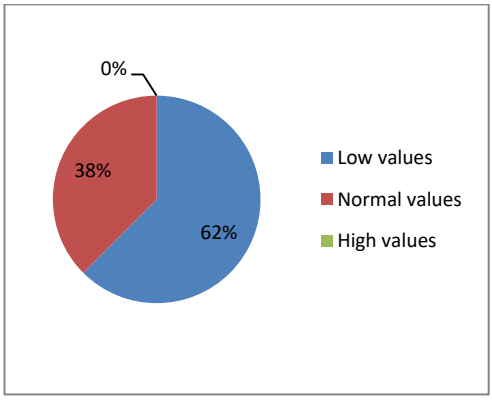

Fig. 5. Biodiversity (Shannon index) group 1

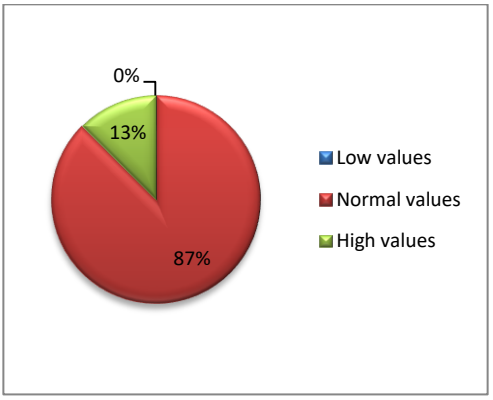

Fig. 6. Biodiversity (Shannon index) group 2

Firmicutes/Bacteroidetes Report

All the participants in group 1 and 2 had normal values.

Butyrate production

In group 1, two participants (25%) had low values, while six participants (75%) had normal values (fig. 7). In group 2, seven participants (87%) had normal values, and one participant (13%) had high values (fig. 8).

Lactate production. In group 1, four participants (50%) had normal values, while the other four (50%) had low values (fig. 9). In group 2, six patients (25%) had normal values, while two subjects (25%) presented high values (fig. 10).

Acetate production. In group 1, five patients (62%) had normal values, while three patients (38%) had high values (fig. 11). In group 2, all subjects had normal values (fig. 12).

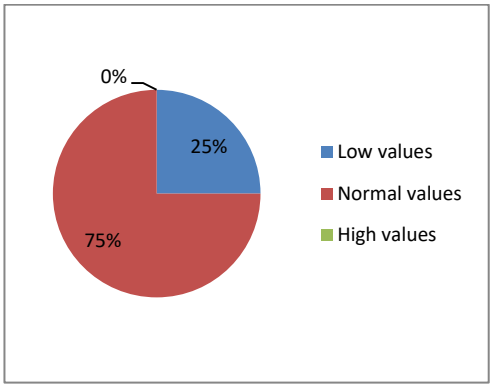

Fig.7. Butyrate production – group 1

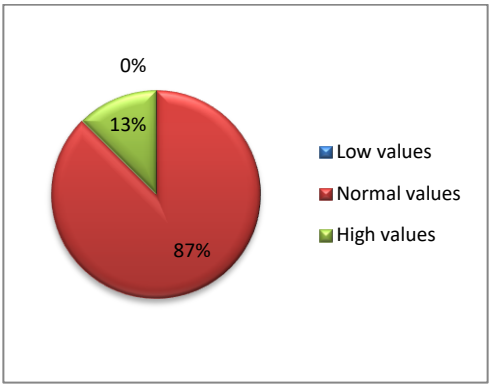

Fig. 8. Butyrate production – group 2

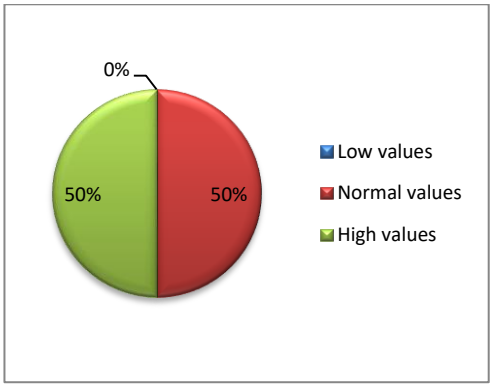

Fig. 9. Lactate production - group 1

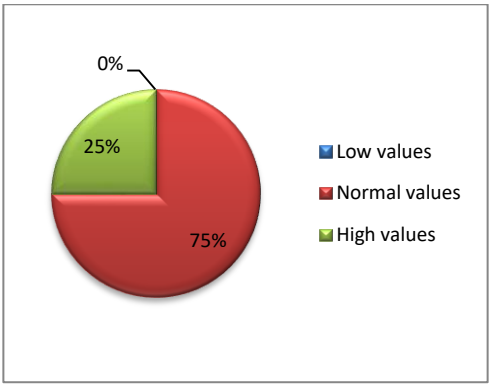

Fig. 10. Lactate production – group 2

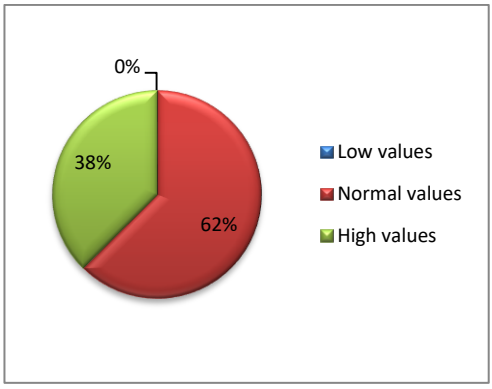

Fig. 11. Acetate production – group 1

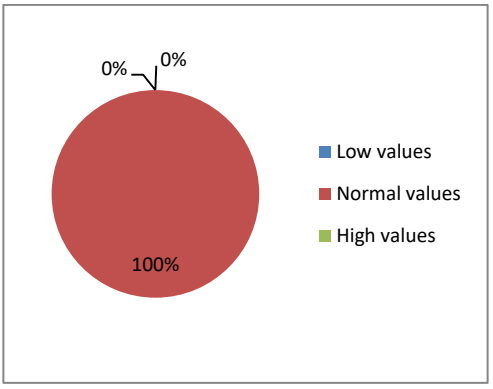

Fig. 12. Acetate production – group 2

Mucin degradation. In group 1, one patient (12%) had low values, six participants (75%) had normal values, while one participant (13%) had high values (fig. 13). In group 2, seven subjects (87%) had normal values, and one (13%) had low value (fig. 14).

Lipopolysaccharide (LPS)-positive bacteria

In group 1, four participants (50%) had normal values, while other four (50%) had high values (fig. 15). In group 2, seven subjects (87%) had normal values, while one (13%) had high values (fig. 16).

Statistics

To test the normality of the distribution, we will apply the Kolmogorov-Smirnov test and the Shapiro-Wilk test and we will interpret the results according to the statistical significance. Conform to the results obtained by the goup 1 in the Kolmogorov-Smirnov test we have an abnormal distribution for Lactate production because $p = 0.001 < 0.05$, for Mucin degradation $p = 0.007 < 0.05$, for Butyrate production $p = 0.001 < 0.05$, and for the rest of the bioindicators the distribution is normal because $p > 0.05$. In the case of the Shapiro–Wilk test, we have an abnormal distribution for Lactate production because $p = 0.004 < 0.05$, for Mucin degradation $p = 0.006 < 0.05$, for Butyrate production $p = 0.003 < 0.05$, and for the rest of the bioindicators the distribution is normal. Since we have a small number of participants, the Shapiro–Wilk test is relevant (tab. I).

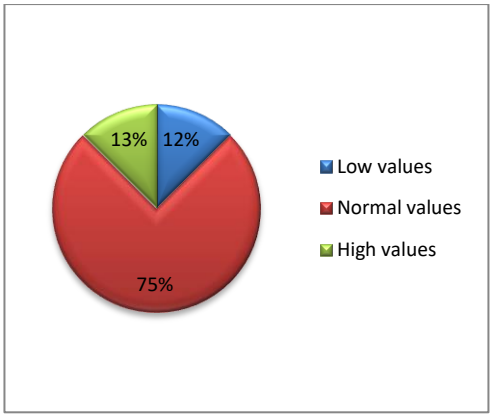

Fig. 13. Mucin degradation – group 1

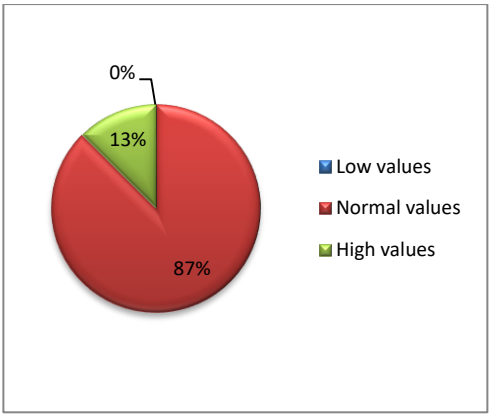

Fig. 14. Mucin degradation – group 2

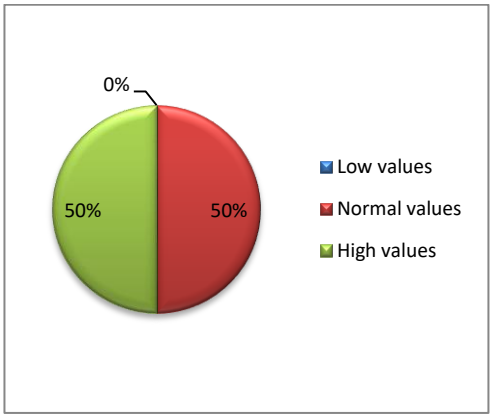

Fig. 15. LPS-positive bacteria – group 1

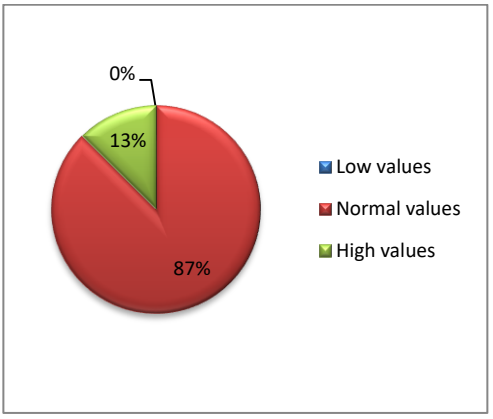

Fig. 16. LPS-positive bacteria – group 2

TABLE I.

Kolmogorov-Smirnov and Shapiro-Wilk normality tests for group1

Tests of Normality						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Fecal pH	.301	8	.051	.782	8	.058
LPS-positive bacteria	.262	8	.114	.840	8	.076
Mucin degradation	.339	8	.007	.736	8	.006
Lactate production	.381	8	.001	.726	8	.004
Acetate production	.262	8	.114	.888	8	.225
Butyrate production	.379	8	.001	.716	8	.003
Firmicutes/Bacteroidetes Report	.162	8	.200*	.897	8	.274
Biodiversity (Shannon index)	.256	8	.130	.886	8	.213
*. This is a lower bound of the true significance. a. Lilliefors Significance Correction						

In the case of Kolmogorov-Smirnov test for second group we have an abnormal distribution for Lactate Production because $p = 0.000 < 0.05$ and for the rest of the bioindicators the distribution is normal because $p > 0.05$. In the case of the Shapiro-Wilk test, we have an abnormal distribution for Lactate production because $p = 0.000 < 0.05$, and for the rest of the bioindicators, the distribution is normal because $p > 0.05$. Since we have a small number of participants, the Shapiro–Wilk test is relevant (tab. II).

After analyzing the Shapiro-Wilk normality test from both groups, we notice that we have a normal distribution for: Acetate production, LPS-positive bacteria, Faecal Ph, Shannon Biodiversity and Firmicutes/Bacteroidetes report and in the case of bioindicators: Lactate production, Mucin degradation and Butyrate production we have an abnormal distribution.

TABLE II.

Kolmogorov-Smirnov and Shapiro-Wilk normality tests for group 2

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Acetate production	.197	8	.200*	.930	8	.520
LPS-positive bacteria	.325	8	.063	.707	8	.053
Mucin degradation	.261	8	.116	.843	8	.082
Firmicutes/Bacteroidetes Report	.260	8	.118	.771	8	.054
Lactate production	.432	8	.000	.498	8	.000
Butyrate production	.168	8	.200*	.966	8	.862
Fecal pH	.250	8	.150	.849	8	.093
Biodiversity (Shannon index)	.125	8	.200*	.984	8	.978
*. This is a lower bound of the true significance. a. Lilliefors Significance Correction						

We will apply the T test in the case of bioindicators with normal distribution and the non-parametric Wilcoxon test in the case of abnormal distribution.

The descriptive statistics of the T test includes the statistical indicators Mean, Standard Deviation and Standard Error Mean (tab. III).

TABLE III.

T-test between group 1 and group 2

Group Statistics					
	Lot	No	Mean	Std. Deviation	Std. Error Mean
Fecal pH	1	8	6.375	.4432	.1567
	2	8	6.500	.3780	.1336
Biodiversity (Shannon index)	1	8	2.713	.6334	.2240
	2	8	2.900	.4899	.1732
LPS-positive bacteria	1	8	1.188	.4324	.1529
	2	8	1.650	2.0424	.7221
Acetate production	1	8	32.13	9.326	3.297
	2	8	31.25	9.192	3.250
Firmicutes/Bacteroidetes Report	1	8	1.350	.1195	.0423
	2	8	1.400	.1414	.0500

The Independent Samples Test compares differences in means of two independent groups in order to determine whether there is statistical evidence that the associated population means are significantly different. The Independent Samples Test is a parametric test (tab. IV).

Levene's subtest is applied to verify homogeneity of variance. The homogeneity of the variance is a basic condition of the T-test. We observe that $p > 0.05$ for all the values of the bioindicators, so the homogeneity of the variance is verified.

In the T-test, all values of $p > 0.05$, so there are no statistically significant differences between the two groups. The same thing can be observed from the values of t are small.

The effect size in the Independent T-test shows how strong are the differences between the groups. The values smaller than 0,20 indicates that the effect size is weak, the values situated between 0,20 and 0,60 indicate that size effect is medium, and the values bigger than 0.60 indicates that the effect size is strong.

For the bioindicators Fecal pH and Biodiversity (Shannon index) we have a medium effect size, in case of LPS-positive bacteria and Acetate production we have a strong effect side and for Firmicutes/Bacteroidetes Report we have a weak effect size (tab. V).

The non-parametric Wilcoxon test is a statistical test that compares two paired groups. The tests essentially calculate the difference between sets of pairs and analyze these differences to establish if they are statistically significantly different from one another. For Mucin degradation it can be seen that $p = 0.208 > 0.05$, so we have no statistical significance between the two groups and the null hypothesis will be retained (tab. VI).

TABLE IV.

Independent Samples Test

Independent Samples Test											
		Levene's Test for Equality of Variances		t-test for Equality of Means							
		F	Sig.	t	df	Significance		Mean Difference	Std. Error Difference	95% CI of the Difference	
						One-Sided p	Two-Sided p			Lower	Upper
Fecal pH	Equal variances assumed	1.167	.298	-.607	14	.277	.554	-.1250	.2059	-.5667	.3167
	Equal variances not assumed			-.607	13.659	.277	.554	-.1250	.2059	-.5677	.3177
Biodiversity (Shannon index)	Equal variances assumed	.895	.360	-.662	14	.259	.519	-.1875	.2831	-.7947	.4197
	Equal variances not assumed			-.662	13.167	.260	.519	-.1875	.2831	-.7984	.4234
LPS-positive bacteria	Equal variances assumed	4.288	.057	-.627	14	.271	.541	-.4625	.7381	-2.0456	1.1206
	Equal variances not assumed			-.627	7.626	.275	.549	-.4625	.7381	-2.1792	1.2542
Acetate production	Equal variances assumed	.006	.940	.189	14	.426	.853	.875	4.630	-9.055	10.805
	Equal variances not assumed			.189	13.997	.426	.853	.875	4.630	-9.055	10.805
Firmicutes/ Bacteroidetes Report	Equal variances assumed	.000	1.000	-.764	14	.229	.458	-.0500	.0655	-.1904	.0904
	Equal variances not assumed			-.764	13.622	.229	.458	-.0500	.0655	-.1908	.0908

TABLE V.

Independent Samples Effect Sizes

		Standardizer[a]	Point Estimate	95% Confidence Interval	
				Lower	Upper
Fecal pH	Cohen's d	.4119	-.303	-1.284	.688
	Hedges' correction	.4357	-.287	-1.214	.650
	Glass's delta	.3780	-.331	-1.314	.675
Biodiversity (Shannon index)	Cohen's d	.5662	-.331	-1.313	.662
	Hedges' correction	.5990	-.313	-1.241	.626
	Glass's delta	.4899	-.383	-1.369	.630
LPS-positive bacteria	Cohen's d	1.4762	-.313	-1.295	.679
	Hedges' correction	1.5617	-.296	-1.224	.642
	Glass's delta	2.0424	-.226	-1.206	.768
Acetate production	Cohen's d	9.260	.094	-.888	1.073
	Hedges' correction	9.796	.089	-.839	1.015
	Glass's delta	9.192	.095	-.889	1.073
Firmicutes/Bacteroidetes Report	Cohen's d	.1309	-.382	-1.365	.615
	Hedges' correction	.1385	-.361	-1.291	.581
	Glass's delta	.1414	-.354	-1.338	.655

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation. Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

TABLE VI.

The non-parametric Wilcoxon test

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The median of differences between Mucin degradation - group1 and Mucin degradation – group 2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.208	Retain the null hypothesis.
a. The significance level is .050. b. Asymptotic significance is displayed.				

For Lactate production the value of p = 0.866 > 0.05, so we have no statistical significance between the two groups, so the null hypothesis will be retained (tab. VII).

For Butyrate production, we see that value of p = 1.000 > 0.05, so we have no statistical significance between the two groups, so the null hypothesis will be retained (tab. VIII).

TABLE VII.

Wilcoxon Test

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The median of differences between Lactate production - group 1 and Lactate production - group 2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.866	Retain the null hypothesis.
a. The significance level is .050. b. Asymptotic significance is displayed.				

TABLE VIII.

Non-parametric Wilcoxon test

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig.[a,b]	Decision
1	The median of differences between Butyrate production - group1 and Butyrate production - group 2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	1.000	Retain the null hypothesis.
a. The significance level is .050. b. Asymptotic significance is displayed.				

DISCUSSION

Because the children from different racial, socioeconomic, and ethnic backgrounds are affected by ASD and because of the high prevalence of ASD in the world, many of the problems associated with ASD cause challenges for society as a whole. Establishing the diagnosis before the age of two and starting psychological and communication therapy help to create quality and can significantly reduce the symptoms associated with autism, due to the plasticity of the brain, which is a characteristic of the early period of development. Balancing the body, especially from a biochemical point of view, can increase the chances of symptom relief and, implicitly, of improving the general state of health. In both groups there are children who present imbalances at the level of the microbiome. This may be influenced by diet and cause further research focusing on the link between diet and the microbiome.

Using a diet, specific nutrients im-

portant for making learning more efficient and increasing social integration cannot be missing. Children with autism spectrum disorder tend to have a limited food repertoire and a greater reluctance to eat food than neurotypical children. Modulation of the gut microbiota in individuals with ASD and gastrointestinal disorders appears to be a promising future medical target. A probiotic approach should act as a means of restoring a healthy microbiota in addition to reducing gut permeability. To confirm the efficiency of probiotic therapy in children with autism spectrum disorders, larger studies with laboratory analyzes of the microbiota are needed for better direction. Further research on the brain-gut-microbiome axis may lead to new methods of identifying gastrointestinal disorders in children with autism spectrum disorders and new treatments for autism spectrum disorder behavior.

Diet plays an important role in determining gut microbial composition and function; therefore, a selective diet can influence the gut microbial community. Overall, the approach to autism needs to be one based on respect, inclusion and support for people with autism and their families. By promoting awareness, understanding and acceptance, we can help create a more inclusive and supportive society for all its members.

CONCLUSIONS

Our research aimed to identify if there is a causal link between intestinal transit disorders such as chronic constipation, the increased level of psychomotor agitation, frequent colds and the presence of an imbalance in the intestinal microbiome bioindicators. After analyzing the values obtained for the bioindicators from the intestinal microbiome, we notice that all the participants in group 1 have values different from the normal ones for at least 1 analyzed indicator, and in the case of group 2, we note that a number of 4 participants have all normal values, and the other 4 participants have at least one value different from the normal one. It can be observed, following the statistical interpretation of the results from test T, that there are no statistically significant differences in terms of microbiome analysis between the two groups participating in the research, $p > 0.05$, a fact that refutes hypothesis 1, namely: *it is assumed that there are statistically significant differences between the results of bioindicators from the intestinal microbiome in children with ASD and in neurotypical children.*

Regarding the connection between constipation and the imbalance at the level of the microbiome, it can be observed that the participants from group 1 present more imbalances at the level of the intestinal microbiome than the participants from group 2, which shows us that hypothesis 2 is confirmed, namely: *it is assumed that the presence of transit disorders such as constipation can be a cause of imbalances in the intestinal microbiome.*

For hypothesis 3, it can be observed, after analyzing the data, that there is a close connection between the problems arising at the level of bioindicators of the intestinal microbiome and the increased psychomotor agitation present in the study participants from group 1, so the hypothesis is thus confirmed, namely: *it is assumed that the disturbance of the profile of intestinal bacteria can influence behavioral manifestations*.

For group 1, we notice that we have a percentage of 75% of participants with enterotype 1 and for group 2 we have a percentage of 100%, that is all participants, with enterotype 1. After the laboratory

analysis, the enterotype of each participant has been identified. In two of the children with ASD, those who are 3 years old, enterotype determination could not be performed, probably due to their young age. We recommend consulting a pediatric gastroenterologist for the implementation of microbiota-targeted therapies, such as probiotics, prebiotics, food supplements. New insights into potential therapeutic interventions that can be used to reduce and treat symptoms associated with autism spectrum disorders can be mentioned.

Our research has showed that people with ASD that have severe symptoms and also transit disorders such as constipation, have an imbalance in the intestinal microbiome.

REFERENCES

1. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Low relative abundances of the mucolytic bacterium Akkermansia muciniphila and Bifidobacterium spp. in feces of children with autism. *Appl Environ Microbiol* 2011; 77(18): 6718-6721.
2. Dan Z, Mao X, Liu Q, *et al*. Altered gut microbial profile is associated with abnormal metabolism activity of Autism Spectrum Disorder. *Gut Microbes* 2020;11(5): 1246-1267.
3. De Angelis M, Piccolo M, Vannini L, *et al.* Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One* 2013; 8(10): e76993.
4. Chernikova MA, Flores GD, Kilroy E, Labus JS, Mayer EA, Aziz-Zadeh L. The Brain-Gut-Microbiome System: Pathways and Implications for Autism Spectrum Disorder. *Nutrients* 2021; 13(12): 4497.
5. Mendive Dubourdieu P, Guerendiain M. Understanding the link between gut microbiota, dietary intake, and nutritional status in children with autism and typical development. *Front Nutr* 2023; 10: 1202948.
6. Kim J. Autism Spectrum Disorder and Eating Problems: The Imbalance of Gut Microbiota and the Gut-Brain Axis Hypothesis. *Soa Chongsonyon Chongsin Uihak* 2024; 35(1): 51-56.
7. Yu Y, Zhao F. Microbiota-gut-brain axis in autism spectrum disorder. *J Genet Genomics* 2021; 48(9): 755-762.
8. Hill AP, Zuckerman KE, Fombonne E. Obesity and autism. *Pediatrics* 2015; 136: 1051-1061.
9. Dawson G, Rogers S, Munson J, *et al*. Randomized, controlled trial of an intervention for toddlers with autism: the Early Start Denver Model. *Pediatrics* 2010;125(1): e17-23.
10. Nadon G, Feldman DE, Dunn W, Gisel E. Association of sensory processing and eating problems in children with autism spectrum disorders. *Autism Res Treat* 2011; 2011: 541926.
11. Karst JS, Van Hecke AV. Parent and family impact of autism spectrum disorders: a review and proposed model for intervention evaluation. *Clin Child Fam Psychol Rev* 2012;15(3): 247-277.
12. Gupta A, Singhal N. Psychosocial support for families of children with autism. *Asia Pacific Disability Rehabilitation Journal.* 2005; 16: 62-83.
13. Tehee E, Honan R, Hevey D. Factors contributing to stress in parents of individuals with autistic spectrum disorders. *Journal of Applied Research in Intellectual Disabilities* 2009; 22: 34-42.
14. Mandell DS, Cao J, Ittenbach R, Pinto-Martin J. Medicaid expenditures for children with autistic spectrum disorders: 1994 to 1999. *J Autism Dev Disord* 2006; 36(4): 475-485.
15. Croen LA, Najjar DV, Ray GT, Lotspeich L, Bernal P. A comparison of health care utilization and costs of children with and without autism spectrum disorders in a large group-model health plan. *Pediatrics* 2006; 118(4): e1203-1211.
16. Martel J. *The great dictionary of diseases and conditions.* Bucharest: Ascendent Publishing House, 2021.