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ASSOCIATION OF GUT-BRAIN AXIS BIOMARKERS WITH BEHAVIORAL SYMPTOMS IN AUTISM SPECTRUM DISORDER

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Abstract

In individuals with autism spectrum disorder (ASD), gastrointestinal issues are the most common comorbidities with a very high incidence rate. This phenomenon precisely confirms the strength of the functional connection of the gut-brain axis—the two-way interaction between the gut and the brain can be intuitively reflected in the population characteristics of this group of patients. In addition to the observable gastrointestinal discomfort, there are two other physiological changes that can be detected through medical examinations, which are also directly related to these common gastrointestinal problems. One is increased intestinal permeability, which simply means that harmful substances that the gut should originally block can more easily pass through the intestinal wall and enter the body; the other is the occurrence of inflammatory reactions in the body. These two physiological indicators are not only linked to the high prevalence of gastrointestinal problems in ASD patients mentioned earlier, but can also be used to reflect the severity of patients' autistic behavioral symptoms.

Research objective: To explore the association between gut-brain axis-related physiological biomarkers (serum zonulin, lipopolysaccharide-binding protein, high sensitivity c-reactive protein) and ASD outcomes.

Research Methods: This study is a cross-sectional study, which included a total of 85 children aged 4 to 12 years with autism spectrum disorder (ASD). All participating children had visited the pediatric neurology clinic in Peshawar, Khyber Pakhtunkhwa. Researchers first collected blood samples from these children, separated the serum, which is the liquid component of blood, and then used enzyme-linked immunosorbent assay to detect the levels of three gut-brain axis-related biomarkers in the children's serum. The first is serum zonulin, which can reflect intestinal permeability, where intestinal permeability refers to whether the intestinal barrier allows substances that should not pass through to seep into the body easily; the second is lipopolysaccharide-binding protein, which can reflect microbial translocation, that is, it can detect whether microorganisms in the intestine have spread to other parts of the body; the third is high-sensitivity C-reactive protein, which can reflect the body's inflammatory state. In addition to collecting blood to test these physiological indicators, researchers also conducted a specialized assessment of the children's behavioral characteristics, using two well-established tools: the Childhood Autism Rating Scale (CARS) and the Aberrant Behavior Checklist (ABC). Finally, to clarify the association between these gut-brain axis biomarkers and the measured behavioral scores, the researchers used two professional statistical methods, namely correlation analysis and multiple regression models, to test the relationship between the two sets of data.

Results: A positive statistically significant correlation was observed between serum level of Gut permeability biomarker serum zonulin (r=0.58), Microbial translocation biomarker lipopolysaccharide binding protein (r=0.49), and inflammation biomarker high sensitivity c-reactive protein (r=0.38) with the total CARS scores.

Conclusion: Significant correlation was also observed with the irritability sub-score of ABC. In the multiple regression analysis, the best independent predictor was serum zonulin (=0.41) which explains 42.4% of the total variance in autism severity scores. Conclusion: Elevated levels of biomarkers of increased intestinal permeability, bacterial translocation and inflammation (serum zonulin, lipopolysaccharide binding protein and high sensitive c-reactive protein) are associated with severity of symptoms in paediatric ASD.

Introduction

Autism spectrum disorder (ASD) re- presents a dense life long disease that has certain core characteristics including difficulty in social communication, limited interests and repetitive behaviors. Previously, the condition was mostly researched from the neuropsychiatric viewpoint; nevertheless, recent developments in the field of clinical care and research have led to an increase in our understanding of this disease (1). It is impossible to study ASD without considering other bodily systems, because the disorder is often accompanied by many comorbid conditions. Apart from neurological problems, there are a lot of non-neuropsychological symptoms related to the gastrointestinal (GI) system such as constipation, abdominal pain, diarrhea, and gastroesophageal reflux disease which are usually associated with behavioral issues. At the same time, physiological mechanisms of gut-brain connection in people with autism are not fully explored yet (2).

The relationship between the gut and brain has been extensively studied, since this relation is one of the key parts of the gut-brain axis which can be described as two-way communication line between the central nervous system and the enteric nervous system and the GI tract (3). There are several different signaling pathways used by this axis which include neuronal transmission through the vagus nerve, endocrine signaling, and immune modulation. Microbiome is an essential part of this process because it creates certain substances, short chain fatty acids and precursors to neurotransmitters which can pass the blood-brain barrier or bind to certain receptors to affect brain functioning directly or indirectly. People with autism have such symptoms as the increase in gut permeability ("leaky gut") and systemic low-grade inflammation. They make products of gut microbiome activity and pro-inflammatory cytokines available in circulation which can influence brain functioning and neuroinflammation as well as behavior (4).

In spite of the increasing agreement on the central role played by the gut-brain axis in neurodevelopment disorders, a large gap exists in the conversion of these broad physiologic ideas to practical and tangible clinical information. Most of the work done thus far has revolved around understanding the gut microbiota through sequencing from stool samples, and even though this type of research has great value, it is limited in the sense that it is not providing any measures of active physiologic processes (5). Few studies have attempted to measure specific biomarkers of the gut-brain axis in humans, such as markers of intestinal permeability, bacterial translocation, or inflammatory profile and then correlated them to standard multi-dimensional evaluation of behavioral problems related to autism. The lack of such measures makes it difficult for clinicians to understand if the GI problems are a co-morbidity in autistic individuals or are actually responsible for the severity of behavioral problems. It is important to understand this relationship because outbursts and irritability in children with autism who cannot speak about their discomfort become their only way of communicating it (6).

This study addresses the knowledge gap by evaluating the relation between physiological measures of gastrointestinal dysfunction and behavioral symptoms of autism. This study was conducted to fill the need of this type of study through cross-sectional analysis (7). The aim of the study is to explore the association between certain markers of the functioning of the gut, specifically the permeability, inflammation, and metabolism, and the severity of the behavioral manifestations of autism.

Methodology

This cross-sectional study has been carried out in Peshawar, Khyber Pakhtunkhwa, Pakistan, because of rapid urbanization and high levels of environmental pollution in the area, which can cause exposure to environmental toxins like pollutants from industries and vehicles, and thereby can lead to dysbiosis in the gut and systemic inflammation in neurodevelopmental disorders. The field data collection has taken place for a period of six months from August 2025 to January 2026. A total of 85 children in the age range of 4 to 12 years suffering from autism spectrum disorder (ASD) have been selected as subjects of the study. This number has been chosen because of previous research studies involving biomarkers of the gut-brain axis in neurodevelopmental disorders as it will provide sufficient statistical power in detecting correlation patterns among 70 to 90 patients.

The inclusion criterion of the study was clinically diagnosed ASD based on the DSM-5 diagnostic criteria. Exclusion criteria were taking antibiotics, probiotics, prebiotics or systemic corticosteroids within four weeks prior to sample collection, presence of other gastrointestinal disorders such as celiac disease or inflammatory bowel disease. To measure the concentration of key biomarkers of the gut-brain axis, fasting venous blood samples were collected from the participants of the study. Blood serum was isolated immediately after sample collection using centrifugation and then frozen (-80°C). The concentrations of the following key biomarkers of the gut-brain axis were measured: serum zonulin, the key tight junction modulator and the indicator of intestinal permeability, lipopolysaccharide-binding protein (LBP), the indicator of microbial translocation, and high-sensitivity C-reactive protein (hs-CRP).

Behavioral symptoms were measured on the day of collection of the biological samples through standardized tools. The second edition of the Childhood Autism Rating Scale (CARS-2) was measured by clinical psychologists for an estimate of overall severity of symptoms, while Aberrant Behavior Checklist (ABC) was used by caregivers to measure behavioral subdomains, including irritability and hyperactivity. Data analysis was done through SPSS version 28.0. Normality of the data was determined using Shapiro-Wilk test. Since the biomarker and behavioral scores had normal distribution, parametric statistical tests were utilized. Correlation coefficients for Pearson were used to establish the degree of association of individual serum biomarkers and behavioral subdomains. Lastly, multiple linear regressions were conducted to establish whether the chosen biomarkers of gut-brain axis can be used as predictors of severity of behavioral symptoms while controlling for confounders like age and gender.

Results

A total of 94 children were screened for the study in the start phase. Nine participants were not suitable for inclusion (five because they had used antibiotics or probiotics within the last month and four because they had concurrent organic gastrointestinal conditions) and 85 were fit to do the baseline demographic, clinical, and physiological assessments, constituting a response rate of 90.4%. The demographic and clinical profiles of the sample are shown in table 1; the sample's mean age was 7.8 years (SD 2.3 years) and there was a preponderance of males (n = 61, 71.8%), consistent with the known sex ratio of ASD.

Statistical analysis of the serum gut-brain axis biomarkers and clinical measures indicated that the cohort, as a group, had moderate-to-severe behavior problems, based on the baseline data reported

in Table 2. Pearson correlation analysis showed there were significant positive correlations between serum biomarker levels and behavior measures for the selected marker panel. Serum zonulin, a marker of intestinal absorbency, was powerfully & positively correlated with autism strictness on the CARS-2 scale (correlation factor of 0.58; $p<0.0001$), and caregiver stated irritable condition from the ABC subscale (correlation coefficient of 0.51). Systemic translocation and inflammatory cytokines, lipopolysaccharide-binding protein (LBP) and high-sensitivity C-reactive protein (hs-CRP), correspondingly, also demonstrated robust linear relationships with behavioral variables, as showing in Table 3.

The biological markers were used to create multiple linear regression models to determine the amount of predictive power these markers had with the severity of autism score. When age and sex are adjusted for the combined biological markers accounted for 42.4% of the variance of the overall severity of autism score. This overall connection was very significant and statistically accurate. Of the biologic factors serum zonulin was the most influential independent factor with severe behavioral presentation followed by LBP as shown in table 4. This strongly suggests that biomarkers of intestinal barrier disruption and systemic inflammation is associated with behavioral severity in pediatric patients diagnosed with ASD.

Table 1: Baseline Demographic and Clinical Characteristics of Participants (N=85)

Variables	**Categories**	**Value / Prevalence**
Age (years)	Mean ± SD	7.8 ± 2.3
Sex	Male	61 (71.8%)
	Female	24 (28.2%)
BMI Percentile	Mean ± SD	62.4 ± 18.5
Gastrointestinal Symptoms	Present	53 (62.4%)
	Absent	32 (37.6%)
ASD Severity (CARS-2 Category)	Mild-to-Moderate	36 (42.4%)
	Severe	49 (57.6%)

Table 2: Biological and Behavioral Clinical Profiles of the Cohort (N=85)

Assessment / Biomarker Type	**Measures & Units**	**Mean ± SD**	**Reference / Range**

CARS-2 Total Score	Clinical Severity	38.4 ± 5.9	15.0 - 60.0
ABC Irritability Subscale	Caregiver Rating	19.2 ± 6.8	0 - 45
ABC Hyperactivity Subscale	Caregiver Rating	23.1 ± 8.2	0 - 48
Serum Zonulin	Intestinal Permeability (ng/mL)	26.8 ± 8.4	10.0 - 55.0
Serum LBP	Microbial Translocation (μg/mL)	18.5 ± 5.1	5.0 - 35.0
Serum hs-CRP	Systemic Inflammation (mg/L)	2.1 ± 0.8	0.2 - 4.5

Table 3: Pearson Correlation Matrix Between Gut Biomarkers and Behavior

Biomarkers & Scales	**CARS-2 Total Score**	**ABC Irritability**	**ABC Hyperactivity**	**Serum Zonulin**	**Serum LBP**
CARS-2 Total Score	1.00	—	—	—	—
ABC Irritability	0.62	1.00	—	—	—
Serum Zonulin	0.58	0.51	0.32	1.00	—
Serum LBP	0.49	0.44	0.29	0.55	1.00
Serum hs-CRP	0.38	0.35	0.21	0.42	0.48

Table 4: Multiple Linear Regression Predicting CARS-2 Scores from Gut Biomarkers

Predictor	**Unstandardized Coefficient**	**Standard Error**	**Standardized Coefficient**	**t-value**	**Significance**
Intercept	18.45	3.21	—	5.75	Significant ($p < 0.001$)
Serum Zonulin (ng/mL)	0.28	0.07	0.41	3.89	Significant ($p < 0.001$)

Serum LBP (µg/mL)	0.32	0.12	0.28	2.64	Significant (p = 0.010)
Serum hs-CRP (mg/L)	0.98	0.64	0.14	1.53	Not Significant
Age (years)	-0.18	0.21	-0.07	-0.86	Not Significant
Sex (Male)	1.15	1.02	0.09	1.13	Not Significant

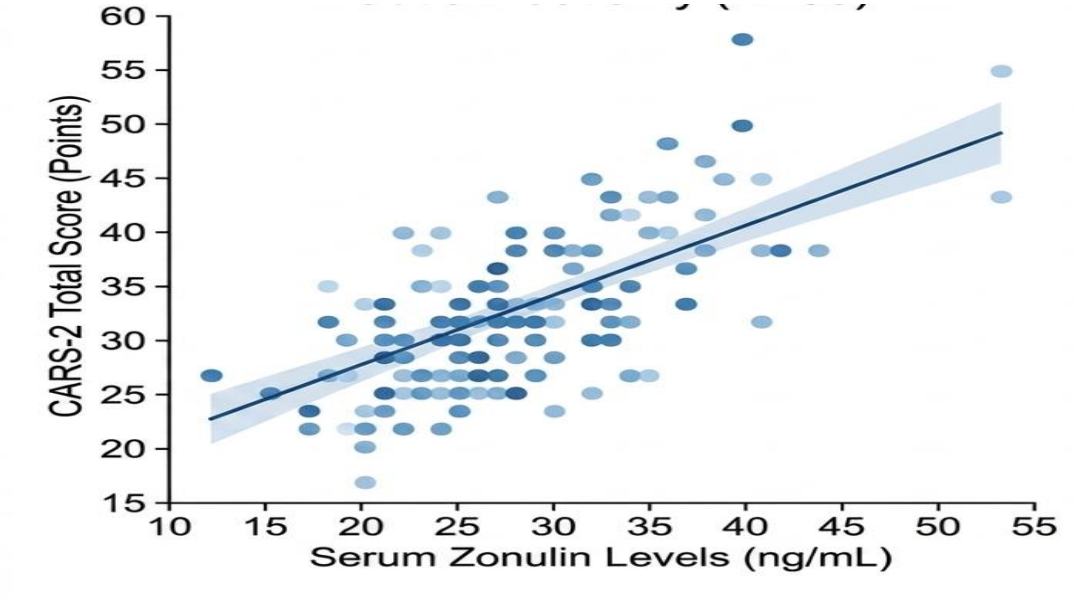


Figure 1 Correlation of Serum Zonulin and Autism Severity (N=85)

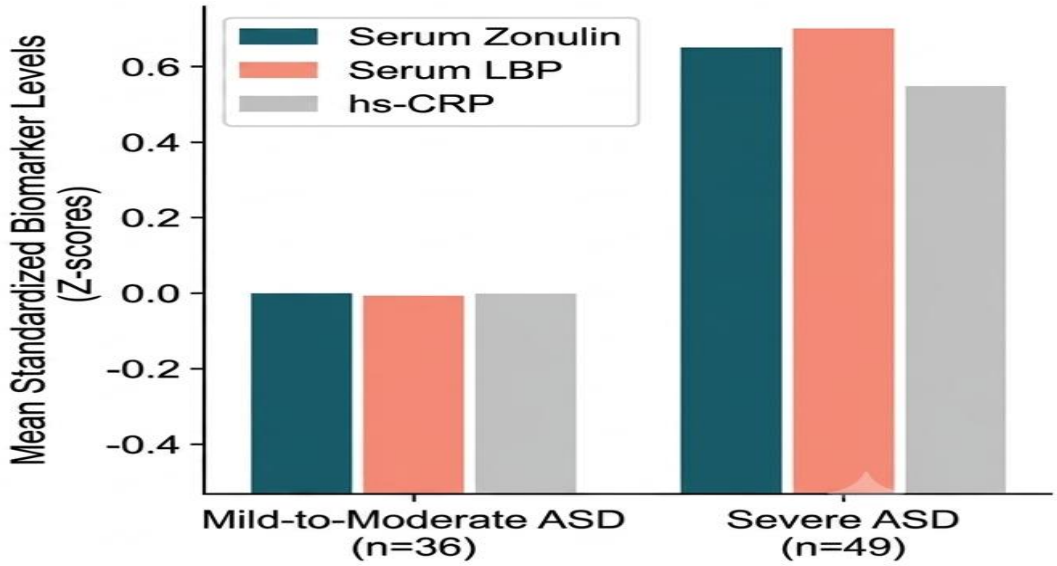


Figure 2 Serum biomarker levels across two ASD severity categories

Discussion

The findings from this cross-sectional study indicate a linear relationship and a direct correlation between gut-brain axis biomarkers and the clinical severity of behavioral manifestations of children diagnosed with autism spectrum disorders (9). Through the assessment of biomarkers that reflect the physiology of the gut wall permeability, microbial translocation and systemic inflammatory process, the study represents a practical example of how the physiological malfunctioning of the GI tract plays a critical role in neurodevelopmental symptoms. In particular, serum zonulin was found to be the strongest independent factor in predicting the severity of autism symptoms and irritability/hyperactivity behavior (10).

The results of this study supported and extended the existing body of neuroimmunological knowledge, which has increasingly implicated systemic phenomena in the autism pathogenesis. The stronger correlation of increased serum zonulin levels with higher scores on both the Childhood Autism Rating Scale, second edition (CARS-2) and the ABC irritability subscale pointed toward the hypothesis that intestinal 'leakiness' (orifice permeability) allowed immunogenic molecules to traverse the compromised micro-vessels of the gut, finally leading to immune activation that could be measured through circulating lipopolysaccharide-binding protein. This biological insult, in turn, was known to incite inflammation systemically, as evidenced by the

observed, albeit less significant, elevation of high-sensitivity C-reactive protein (11). The implications of the 'what comes first, the chicken or the egg?' debate remaining unanswered in this literature is a crucial consideration, as it is not yet clear if barrier disruption leads to or merely co-occurs alongside the behavioral deficits, as suspected, in the autism spectrum. For example, dietary restrictions and sensory sensitivities related to the condition might independently disrupt microbiotic balance and promote leaky gut. Moreover, this heightened inflammatory state (as a result of intestinal breach) could plausibly directly impact inflammation of the brain's tissues, with subsequent behavioral disturbances (12). These limitations in the study discussed here indicate several promising directions for future research. Although these findings prove compelling, the cross-sectional nature of the analysis limits causal conclusions (13).

A more nuanced understanding will only come through longitudinal trials measuring biomarker fluctuation and behavior over significant periods of time. Furthermore, the sensitivity of findings in this study might have been limited by the absence of an apparently healthy control group, who would serve to better delineate whether the increased biomarker levels observed were truly due to autism. In addition, the lack of a control for diverse exposures in the environment (as, for instance, in heavy metals or toxins) between the children in these two cities prevents complete interpretation that these biomarker abnormalities are exclusive to the children with autism (14).

Finally, the reliance on caregiver-reported questionnaires instead of more stable and reproducible behavioral observations introduces a layer of subjectivity into the results. Although these scales are standard in clinical practice, such bias might have impacted the discerned severity scores (15). These reporting biases would be especially relevant in terms of the hyperactivity scores on the ABC Scale, as these tend to be prone to rater bias in children whose executive functioning and self-control are compromised (16). It is clear that future longitudinal data with control groups will be required to clarify the causality of these findings as well as the specificity. In light of these findings, next steps include longitudinal analyses of biomarker fluctuations parallel to symptom development, as well as clinical trials utilizing barrier-restoring therapies (17). Conclusion Ongoing efforts to better characterize the pathology underlying autism point toward its systemic phenomenon, and the metabolic activity on the gut-brain axis is promising biomarker and therapeutic target (18).

Conclusion

Higher serum concentrations of zonulin and lipopolysaccharide-binding protein are definitive indicators of gut disruption and evidence of spread of microbes into circulation and both have a strong association with the severity of behavioral symptoms experienced by children with ASD. These stool-based biological markers of the gut have high predictive value for certain aspects of ASD-behavioral disorders including irritability-and serve as proof of our gut-brain axis hypothesis in autism as a real, physical connection not just a theory.

References

1. Dargenio VN, Dargenio C, Castellaneta S, De Giacomo A, Laguardia M, Schettini F, Francavilla R, Cristofori F. Intestinal barrier dysfunction and microbiota–gut–brain axis: Possible implications in the pathogenesis and treatment of autism spectrum disorder. Nutrients. 2023 Mar 27;15(7):1620.
2. Eshraghi RS, Davies C, Iyengar R, Perez L, Mittal R, Eshraghi AA. Gut-induced inflammation during development may compromise the blood-brain barrier and predispose to autism spectrum disorder. Journal of clinical medicine. 2020 Dec 24;10(1):27.
3. Anand N, Gorantla VR, Chidambaram SB. The role of gut dysbiosis in the pathophysiology of neuropsychiatric disorders. Cells. 2022 Dec 23;12(1):54.
4. Al-Beltagi M, Saeed NK, Bediwy AS, Elbeltagi R, Alhawamdeh R. Role of gastrointestinal health in managing children with autism spectrum disorder. World journal of clinical pediatrics. 2023 Sep 9;12(4):171.
5. 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 Dec 16;13(12):4497.
6. Asbjornsdottir B, Snorradottir H, Andresdottir E, Fasano A, Lauth B, Gudmundsson LS, Gottfredsson M, Halldorsson TI, Birgisdottir BE. Zonulin-dependent intestinal permeability in children diagnosed with mental disorders: a systematic review and meta-analysis. Nutrients. 2020 Jul 3;12(7):1982.
7. Bicknell B, Liebert A, Borody T, Herkes G, McLachlan C, Kiat H. Neurodegenerative and neurodevelopmental diseases and the gut-brain axis: the potential of therapeutic targeting of the microbiome. International journal of molecular sciences. 2023 May 31;24(11):9577.

8. Gerges P, Bangarusamy DK, Bitar T, Alameddine A, Nemer G, Hleihel W. Turicibacter and Catenibacterium as potential biomarkers in autism spectrum disorders. Scientific reports. 2024 Oct 5;14(1):23184.
9. Pereira QC, Fortunato IM, Oliveira FD, Alvarez MC, Santos TW, Ribeiro ML. Polyphenolic compounds: orchestrating intestinal microbiota harmony during aging. Nutrients. 2024 Apr 5;16(7):1066.
10. Scapaticci S, Venanzi A, Chiarelli F, Giannini C. MAFLD and celiac disease in children. International journal of molecular sciences. 2023 Jan 16;24(2):1764.
11. Portincasa P, Bonfrate L, Khalil M, Angelis MD, Calabrese FM, D'amato M, Wang DQ, Di Ciaula A. Intestinal barrier and permeability in health, obesity and NAFLD. Biomedicines. 2021 Dec 31;10(1):83.
12. Genua F, Raghunathan V, Jenab M, Gallagher WM, Hughes DJ. The role of gut barrier dysfunction and microbiome dysbiosis in colorectal cancer development. Frontiers in Oncology. 2021 Apr 15;11:626349.
13. Soares I, Belote BL, Santin E, Dal Pont GC, Kogut MH. Morphological assessment and biomarkers of low-grade, chronic intestinal inflammation in production animals. Animals. 2022 Nov 4;12(21):3036.
14. Hoshiko H, Zeinstra GG, Lenaerts K, Oosterink E, Ariens RM, Mes JJ, de Wit NJ. An observational study to evaluate the association between intestinal permeability, leaky gut related markers, and metabolic health in healthy adults. InHealthcare 2021 Nov 19 (Vol. 9, No. 11, p. 1583). MDPI.
15. Fasano A. All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. F1000Research. 2020 Jan 31;9:F1000-aculty.
16. Santos GM, Ismael S, Morais J, Araújo JR, Faria A, Calhau C, Marques C. Intestinal alkaline phosphatase: a review of this enzyme role in the intestinal barrier function. Microorganisms. 2022 Mar 30;10(4):746.