GMR
The Original

ASSOCIATION OF PLASMA AMMONIA WITH BEHAVIORAL SEVERITY IN AUTISM SPECTRUM DISORDER

Dr Nissy Susan Varughese[1], Dr Manju George Elenjickal[2], Dr Vikram Gowda N R[3], Dr Amrutha Mary Zachariah[4]

[1]Assistant Professor of Physiology, Pushpagiri Institute Of Medical Sciences & Research Centre , Thiruvalla, ORCID ID: https://orcid.org/0009-0006-9009-4718, nissysusanamith@gmail.com
[2]Professor of Pediatrics, And in charge of Pratheeksha CDC, Pushpagiri Institute Of Medical Sciences and Research centre ,Thiruvalla, majugeorge282@gmail.com
[3]Professor of Physiology, Vice Principal, Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla
https://orcid.org/0000-0003-1374-6162, vikramgowda@ymail.com
[4]Professor and HOD of Physiology, Pushpagiri Institute Of Medical Sciences and Research Centre ,Thiruvalla

ABSTRACT:
Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by persistent deficits in communication, social interaction, and behavior. While its behavioral features are well documented, the biochemical correlates of symptom severity remain underexplored. Plasma ammonia, a neurotoxic metabolite, may contribute to ASD pathophysiology by disrupting neuronal signaling and brain metabolism.
Objectives: This study aimed to measure plasma ammonia levels in children diagnosed with ASD and to evaluate the association between ammonia concentration and the severity of autistic behaviors using the Childhood Autism Rating Scale (CARS).
Methods: A cross-sectional observational study was conducted over 10 months at a tertiary pediatric institution in Kerala, India. Fifty-three children aged 3–7 years with a confirmed diagnosis of ASD (DSM-5 criteria) were retrospectively analyzed. Plasma ammonia concentrations were measured using the Beckman Coulter AU680 analyzer, and behavioral severity was assessed using CARS. Pearson correlation analysis was used to determine associations between variables.
Results: Plasma ammonia levels were elevated in 27 of 53 children (50.9%). A moderate, statistically significant positive correlation was observed between plasma ammonia levels and CARS scores ($r = 0.31$, $p = 0.024$). Ammonia levels did not show significant associations with age or gender.
Conclusion: Elevated plasma ammonia levels may serve as a simple, low-cost, and accessible biochemical marker of behavioral severity in children with ASD. These findings support the integration of metabolic screening with behavioral assessment to facilitate early detection and personalized intervention strategies.

KEYWORDS: Ammonia, Autism Spectrum Disorder, Behavioral Severity, Hyperammonemia, Metabolic Biomarkers.

INTRODUCTION

Autism spectrum disorder (ASD) is a complicated neurodevelopmental illness that is typified by persistent social communication difficulties and restricted, repetitive behavioral patterns. Over the past 20 years, it has become more widespread globally, and extensive studies have been done on its genesis, diagnosis, and potential therapy targets. While the behavioral aspect of ASD is widely recognized, less is known about the disorder's biochemical foundations, particularly about metabolic indicators such as blood ammonia, which may provide hints about neurotoxic mechanisms affecting behavior and brain development.

A neuroactive chemical called ammonia is produced during the metabolism of proteins. In healthy individuals, ammonia can be converted by the liver to urea, which the kidneys subsequently eliminate. However, hyperammonemia, or elevated ammonia levels, can cause serious brain damage. Measuring ammonia has long been crucial in evaluating metabolic diseases and hepatic encephalopathy [1]. In addition to liver disease, its use has been expanded to include neurological and psychiatric conditions such as epilepsy and mood disorders [2]. The development of ammonia detection, including point-of-care sensors, has made it possible to monitor this metabolite more accurately in a variety of clinical conditions [3]. Furthermore, elevated blood ammonia levels have emerged as prognostic factors in emergency medicine. For example, studies have demonstrated its utility in predicting neurological outcomes following out-of-hospital cardiac arrest [4]. Nevertheless, variations in sampling site, storage conditions, and patient comorbidities continue to affect measurement reliability, particularly in pediatric populations where metabolic and nutritional factors are highly variable [5]. Importantly, even mild elevations in ammonia have been associated with neurocognitive disturbances and impaired psychomotor performance in non-hepatic conditions, emphasizing ammonia's broader neurological relevance [6].

Despite these insights, the investigation of blood ammonia concentrations specifically in children with ASD remains limited. Children with autism frequently exhibit gastrointestinal disturbances, selective dietary habits, altered gut microbiota, and possible mitochondrial dysfunction, all of which can influence nitrogen metabolism and ammonia production [7,8]. These metabolic irregularities may predispose certain subgroups of children with ASD to higher systemic ammonia levels, potentially exacerbating neurobehavioral symptoms [9,10].

Ammonia at high concentrations exerts neurotoxic effects that impair neuronal signaling, alter energy metabolism, and disrupt the balance of neurotransmitters within the central nervous system. The blood–brain barrier is readily permeable

to ammonia, allowing it to reach the brain, where it disturbs astrocyte function, interferes with the conversion of glutamate to glutamine, and induces oxidative stress and mitochondrial dysfunction [11]. These alterations can lead to cognitive deficits, behavioral disturbances, and motor impairments, even in the absence of hepatic disease. In children with autism spectrum disorder (ASD), such disruptions may aggravate existing neurodevelopmental vulnerabilities, contributing to irritability, hyperactivity, and communication difficulties [12]. Emerging evidence indicates that metabolic dysregulation, including elevated ammonia levels, may serve as a peripheral indicator of impaired brain bioenergetics in ASD, underscoring the need to examine ammonia not merely as a metabolic byproduct but as a potential contributor to neurobehavioral pathology [13].

In parallel, ASD research has increasingly explored its multifactorial etiology, encompassing genetic, epigenetic, environmental, and immunological factors [14]. Mitochondrial dysfunction, oxidative stress, and immune dysregulation are now recognized as significant neurodevelopmental risk factors in ASD pathogenesis [15]. These biological pathways intersect with metabolic biomarkers such as ammonia, particularly during early brain development.

Clinical pediatric guidelines stress the importance of early ASD diagnosis and intervention, given the high neural plasticity in early life that presents a crucial therapeutic window [16]. Meanwhile, neurobiological studies have identified specific brain structures and circuits implicated in ASD, enhancing our understanding of how biochemical imbalances may translate into functional deficits [17]. In clinical practice, however, challenges remain in managing behavioral symptoms and associated comorbidities, such as dental anxiety and feeding difficulties, which can further aggravate metabolic abnormalities [18].

Prenatal and perinatal risk factors such as infections, metabolic disorders, nutritional deficiencies, teratogen exposure, and maternal dietary imbalances are increasingly acknowledged as potential contributors to ASD [19]. These factors may directly or indirectly affect ammonia metabolism via liver function, gut microbiota changes, or mitochondrial activity.

Although a strong theoretical link exists between ammonia metabolism and neurodevelopment, there is a marked lack of empirical evidence on blood ammonia levels in children with ASD. Most prior research has been in hepatic or toxicological contexts, with minimal exploration of how hyperammonemia might correlate with behavioral severity in ASD populations.

Objectives of the study

The objectives of the present study were twofold. First, the study aimed to measure and categorize plasma ammonia levels in a cohort of children clinically diagnosed with autism spectrum disorder (ASD). Second, it sought to evaluate the association between blood ammonia concentrations and the severity of behavioral symptoms, as assessed using the Childhood Autism Rating Scale (CARS).

Rationale and Significance of the Study

This study is one of the few in India, and among the very few worldwide, to empirically examine the plasma ammonia profile in children with ASD. It employs standardized biochemical measurement protocols and validated clinical behavioral scales to explore a potentially novel biomarker for behavioral severity.

Understanding the metabolic profile of children with ASD could bridge the gap between behavioral manifestations and underlying biological mechanisms, paving the way for targeted interventions. If elevated ammonia is shown to correlate with symptom severity, it could serve as a cost-effective, accessible biomarker to support early diagnosis and personalized treatment planning. Moreover, these findings may stimulate further multidisciplinary research at the intersection of neurodevelopment, biochemistry, and clinical pediatrics.

METHODOLOGY

Study Design

A cross-sectional observational strategy was used in this study to measure blood ammonia levels in children with ASD diagnosis.

Study Setting and Duration

The study was conducted at a Tertiary Care Centre, Kerala, in the Department of Pediatrics in collaboration with the Department of Physiology. Data was gathered and examined over the 10 months.

Ethical Considerations

Ethical clearance was obtained from the Institutional Ethics Committee before commencing the study.To the greatest extent possible, participant anonymity was maintained, and no personally identifiable information was disclosed.

Study Population

Children diagnosed with ASD in a clinical setting according to the DSM-5 were included as the study population in this study (qualified developmental pediatricians or psychiatrists confirmed the diagnosis). Targeted children were those at an early age of development, and this stage is characterized by high plasticity in the number of neurons, which also corresponds with variability in metabolic processes taking place.

Selection Criteria

Children between the ages of three and seven met the inclusion requirements for the study: a confirmed diagnosis of ASD by qualified clinicians based on the requirements contained in the DSM-5, a recorded plasma ammonia level in their medical history, and complete clinical and behavioral data, including CARS scores. The exclusion criteria included children with a known hepatic or renal dysfunction, a history of taking medications that are known to interfere with ammonia metabolism, including adriamycin and valproic acid, in cases where crucial biochemical or behavioral information is not complete or lost, and patients with reported genetic disorders and chromosomal abnormalities.

Sample size calculation

The sample size was calculated to estimate the proportion of children with ASD presenting with elevated plasma ammonia levels, with a 95% confidence level and 80% power. Based on previous pediatric metabolic studies reporting approximately 50% prevalence of hyperammonemia among ASD subgroups, the expected proportion (p) was taken as 0.50 to maximize sample precision. The absolute allowable error (d) was set at 0.135. Using the formula for estimating a single population proportion [20]:

$$n = \frac{Z_{1-\alpha/2}^2 \times p(1-p)}{d^2}$$

Where,

$Z_{1-\alpha/2} = 1.96$ (for 95% confidence level),

$p = 0.50$,

$$d = 0.135$$

Substituting the values:

$$n = (1.96)^2 \times 0.5(0.5)/(0.135)^2 = 52.8$$

Thus, the minimum required sample size was 53 participants, which was achieved in this study. An 80% power was adopted to ensure adequate precision in the prevalence estimate. The calculation aligns with the recommendations of Lwanga and Lemeshow (1991) [20] and was cross-verified using the approach by Sharma et al. (2020) [21] for observational designs.

Sampling method

Consecutive (total enumeration) sampling was applied to all eligible medical records during the study window (January–October 2018) at the tertiary care center. All children aged 3–7 years with clinician-confirmed ASD (DSM-5) and complete data for plasma ammonia and CARS were included. Exclusion criteria were hepatic or renal dysfunction, use of medications affecting ammonia metabolism, genetic or chromosomal disorders, and incomplete records. This census approach minimized selection bias by attempting to include every eligible case available within the defined time frame and setting [21].

Variables Studies

The primary variable was plasma ammonia concentration, expressed in micromoles per liter (µmol/L) and converted to micrograms per milliliter (µg/mL) under pediatric practice standards. Secondary variables included age (in years), gender, CARS score, behavioral symptoms (e.g., irritability, aggression, speech delay), and comorbid conditions such as epilepsy or neurodevelopmental regression.

Instruments and Tools

The assessment of behavioral and clinical variables was conducted through a review of patient files and clinical records. Behavioral severity was measured using the CARS. Biochemical data were sourced from laboratory reports. Plasma ammonia levels were estimated using the Beckman Coulter AU680 autoanalyzer, which is a fully automated and validated biochemical analyzer. Blood samples were collected under standard conditions and processed by centrifugation to separate plasma, which was then analyzed promptly to minimize pre-analytical variability. According to institutional guidelines, plasma ammonia levels below 70 µmol/L (approximately 1.2 µg/ml) were considered to be within the normal pediatric range.

Data Collection Procedure

Data were collected retrospectively from patient case records maintained in the pediatric department. A structured checklist was utilized to extract information on demographics, clinical symptoms, behavioral assessments, and biochemical results. Each record was de-identified and assigned a unique study code for data entry. All collected data were compiled into Microsoft Excel spreadsheets and reviewed for completeness and consistency before analysis.

Statistical Analysis

Descriptive statistics, including mean, standard deviation, minimum, maximum, and range, were used to characterize continuous variables like age, plasma ammonia level, and CARS scores. Categorical variables were described using percentages and frequencies. Pearson correlation analysis was used to determine the strength and direction of linear connections between ammonia levels and certain variables, such as age and CARS score. A p-value of less than 0.05 was considered to be statistically significant. Pie charts and other visual aids were also used to show how individuals' high vs normal ammonia levels were distributed. To investigate data patterns and find outliers, box plots and scatter plots were employed.

RESULTS

Demographic and Clinical Characteristics

A total of 53 children clinically diagnosed with autism spectrum disorder (ASD) were included in the final analysis. The participants' average age was 3.58 ± 0.93 years, with most children falling within the 3–4-year age range. The gender distribution showed a slight predominance of males, consistent with the global epidemiology of ASD, comprising 28 males (52.8%) and 25 females (47.2%).

Behavioral severity, as assessed using the Childhood Autism Rating Scale (CARS), ranged from 30 to 40, with a mean score of 34.04 ± 2.67. These values indicate that most children in this cohort exhibited mild to moderate autism, with no participants scoring within the severe range. This may reflect a selection bias toward children with milder symptom presentations or those more likely to seek early intervention.

A summary of continuous variables, including age, plasma ammonia, and CARS scores, is provided in Table 1.

Table 1. Descriptive Statistics of Key Variables (N = 53)

Variable	Mean	Standard Deviation	Minimum	Maximum
Age (years)	3.58	0.93	3.0	7.0
Ammonia (µg/mL)	2.45	8.07	0.6	60.0
CARS Score	34.04	2.67	30.0	40.0

Distribution of Plasma Ammonia Levels

A histogram of plasma ammonia levels (Figure 1) demonstrated a positively skewed distribution, with most values clustered between 1.0 and 2.5 µg/mL. A few outliers exhibited markedly elevated ammonia concentrations approaching 60 µg/mL, suggesting the presence of a metabolically distinct subgroup within the ASD cohort.

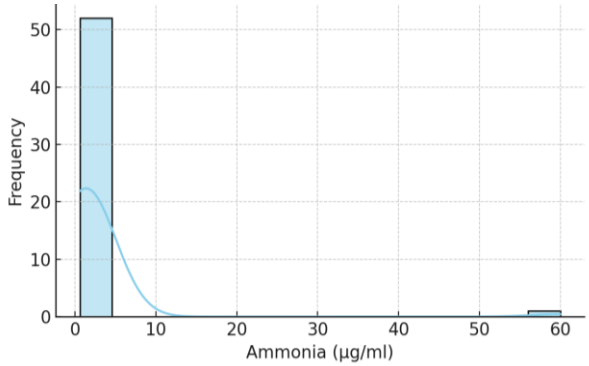

Figure 1. Distribution of Plasma Ammonia Levels

Classification Based on Ammonia Status

Children were classified into two groups using an institutional reference cutoff of 1.2 µg/mL for plasma ammonia. Of the 53 children, 27 (50.9%) exhibited elevated ammonia levels, while 26 (49.1%) had normal levels.

This nearly equal distribution highlights the metabolic heterogeneity among children with ASD and aligns with emerging evidence suggesting altered biochemical profiles in certain subgroups. The classification summary is presented in Table 2.

Table 2. Plasma Ammonia Classification Based on 1.2 µg/mL Threshold

Ammonia status	Frequency	Percentage (%)
Elevated	27	50.9
Normal	26	49.1

Gender-Based Comparison

A boxplot comparison of plasma ammonia levels between sexes (Figure 2) showed that males had a slightly higher median ammonia concentration than females, although the variability within each group was substantial. The overlapping interquartile ranges indicate that this difference was not statistically significant.

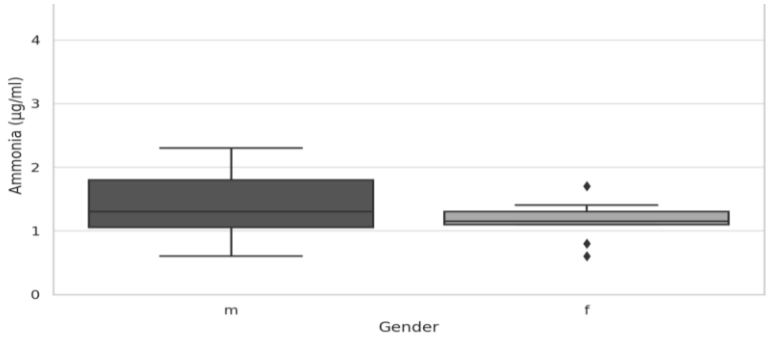

Figure 2. Ammonia Levels by Gender

Correlation Between Ammonia and Clinical Variables

To examine potential associations between biochemical and behavioral measures, Pearson correlation analysis was conducted. The results showed a negative and very weak correlation between plasma ammonia levels and age ($r = -0.08$, $p = 0.555$), indicating no statistically significant relationship between the two variables. In contrast, a moderate and statistically significant positive correlation was observed between plasma ammonia levels and CARS scores ($r = 0.31$, $p = 0.024$), suggesting that children with higher ammonia concentrations tended to exhibit more pronounced behavioral symptoms associated with autism spectrum disorder (ASD). These findings imply that elevated plasma ammonia may be linked to increased behavioral severity, even though it appears to be independent of age (Table 3).

Table 3. Pearson Correlation Analysis Between Ammonia and Other Variables

Variable Pair	Correlation Coefficient (r)	p-value	Significance
Ammonia vs. Age	-0.08	0.555	Not Significant (NS)
Ammonia vs. CARS Score	0.31	0.024	Significant

Visual Representation of Correlation Patterns

Figure 3 illustrates the relationship between ammonia levels and age, showing random dispersion without any discernible trend, confirming the lack of correlation. The absence of a visible trend line and random dispersion of data points reflects the lack of association between age and ammonia levels.

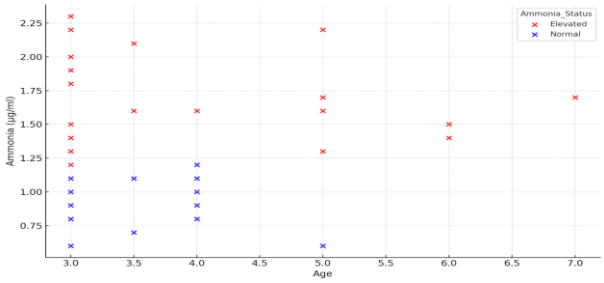

Figure 3. Ammonia vs Age

In contrast, Figure 4, depicting ammonia levels against CARS scores, reveals a mild upward trend, particularly among children with elevated ammonia levels, supporting the observed positive association between metabolic abnormalities and behavioral severity.

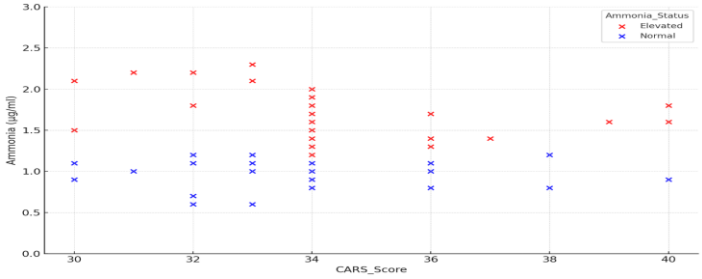

Figure 4. Ammonia vs CARS Score

Additional Observations and Clinical Implications

Visual inspection of outliers identified a few children with extremely elevated ammonia levels (>10 µg/mL), significantly deviating from the group mean. These cases may warrant further metabolic or genetic evaluation to rule out urea cycle defects, inborn errors of metabolism, or subclinical hepatic dysfunction.

Although gender differences were not statistically significant, future research with larger, stratified samples may clarify whether sex-specific biochemical variations contribute to ASD pathophysiology.

DISCUSSION

The present research examined the correlation between the severity of behavior as assessed by CARS and the plasma ammonia levels in children with ASD. The findings have important ramifications for the metabolic profile of children with ASD and could be useful as a therapeutic biomarker. Hyperammonemia, defined as a high quantity of ammonia in the plasma exceeding 1.2 µg/mL, was present in approximately 50.9% of the children studied. The ammonia concentration ranged between 0.6 and 60.0 µg/mL, with a mean of 2.45 ± 8.07 µg/mL. This wide range suggests considerable metabolic diversity among children with ASD. Notably, the levels of plasma ammonia and CARS scores showed a moderate but statistically significant positive correlation ($r = 0.31$, $p = 0.024$), indicating that children with higher ammonia levels were more likely to exhibit more severe autistic behaviors.

This correlation does not imply causation, but it supports the hypothesis that neurotoxic metabolites such as ammonia may influence or exacerbate behavioral symptoms in ASD. The absence of a significant correlation between age and ammonia levels ($r = -0.08$, $p = 0.555$) suggests that other factors, including mitochondrial function, dietary habits, or subclinical hepatic dysfunction, may contribute to elevated ammonia levels during early childhood, rather than chronological age itself. The comparison between males and females did not reveal statistically significant differences, indicating that metabolic variations related to sex are unlikely to be a dominant factor in early childhood ASD, at least in terms of plasma ammonia concentration. Extreme outliers (ammonia >10 µg/mL) are clinically relevant, as they may warrant additional diagnostic evaluation for urea cycle disorders, mitochondrial dysfunction, or other inborn errors of metabolism, which might remain undetected due to overlapping behavioral symptoms.

These results align with contemporary neurobiological models that identify metabolic imbalance and oxidative stress as key contributors to ASD pathophysiology. Neurodevelopmental impairments have been associated with mitochondrial dysfunction, nitrogen metabolism abnormalities, and disruptions in the ROS/NF-κB pathway [17]. Additionally, brain regions such as the prefrontal cortex and amygdala, both implicated in ASD, are highly susceptible to ammonia-induced

neurotoxicity, which may explain the observed link between metabolic alterations and behavioral outcomes [17,18]. Recent studies have also highlighted the relationship between metabolic biomarkers and immune-inflammatory processes in ASD, suggesting that ammonia may act as a systemic regulator of neural activity [18,19]. Moreover, growing evidence indicates that the metabolic profile of children with ASD is shaped by prenatal factors such as maternal health, nutrition, and environmental exposures [19].

However, the diagnosis and management of ASD continue to evolve in India, where clinical and research challenges persist. The current findings provide a quantifiable biological correlate that could enhance behavioral diagnostic approaches and facilitate a more comprehensive assessment of ASD. Some studies also suggest that metabolic markers like lactate and ammonia may respond to exercise-based or lifestyle interventions, highlighting the potential of non-pharmacological strategies as adjunctive therapies [21]. Given the variability in ASD diagnosis related to ethnicity, access to care, and socioeconomic factors, integrating objective biomarkers such as plasma ammonia could reduce subjective bias in diagnosis [22]. The present study contributes to ongoing initiatives in India aimed at improving early diagnosis and individualized treatment for children with ASD [23]. As the field moves toward precision medicine [24], these results lay a foundation for translational research. Furthermore, in cases where children with ASD have a history of developmental trauma, neglect, or institutionalization, incorporating objective metabolic markers such as ammonia may improve diagnostic accuracy in complex clinical contexts [25].

The observed relationship between rising ammonia levels and higher CARS scores suggests that ammonia could serve as a potential indicator of behavioral severity in ASD. If confirmed in larger and more diverse cohorts, plasma ammonia measurement could become part of standardized screening protocols for children undergoing neurodevelopmental assessment. Clinically, identifying children with elevated ammonia levels could guide specific nutritional or pharmacological interventions aimed at reducing systemic ammonia, such as dietary protein modulation, gut microbiota regulation, or mitochondrial support therapy. In resource-limited settings, such biochemical screening could serve as a cost-effective adjunct to traditional behavioral evaluation methods.

This study has several limitations. First, its cross-sectional design precludes causal inference and limits the ability to assess temporal changes in ammonia levels. Second, the absence of a neurotypical control group restricts direct comparison. Third, the analysis did not adjust for potentially influential factors such as medication use, gastrointestinal comorbidities, or dietary patterns. The relatively small sample size and single-center setting may also affect the generalizability of the findings. Additionally, pre-analytical variables such as fasting duration and sample handling time were not uniformly recorded, which could have contributed to measurement variability even with standardized equipment.

Future research should employ larger, multi-center, longitudinal designs with matched neurotypical controls to validate these findings. Tracking ammonia fluctuations over time and their responsiveness to therapeutic interventions could provide valuable insights into treatment mechanisms. Exploring the gut–brain axis, particularly the role of gut dysbiosis in ammonia generation, may further elucidate the metabolic underpinnings of ASD and support combined therapeutic strategies. Integrating ammonia analysis into multi-omics platforms (genomics, metabolomics, and proteomics) could clarify its role within the broader ASD biomarker framework. Establishing clinical cutoffs and identifying ASD subtypes based on metabolic profiles may eventually lead to more targeted, mechanism-driven interventions and improved outcomes for affected children.

CONCLUSION

This study is among the first empirical investigations in India to examine the relationship between plasma ammonia levels and behavioral severity in children with autism spectrum disorder (ASD). The findings demonstrate a moderate and statistically significant correlation ($r = 0.31$, $p = 0.024$) between elevated plasma ammonia concentrations and higher Childhood Autism Rating Scale (CARS) scores. The observation that over half of the participants (50.9%) exhibited hyperammonemia underscores the metabolic variability within the ASD population. These results highlight the potential of plasma ammonia as a simple, accessible biomarker that could complement behavioral assessments in clinical practice. Integrating biochemical screening with behavioral evaluation may enhance diagnostic precision, particularly in resource-limited settings, where early identification of children requiring specialized metabolic or dietary interventions could improve outcomes. The study also contributes to a deeper understanding of neurobiological heterogeneity in ASD, supporting the view that behavioral symptoms may reflect underlying metabolic imbalances. Moving forward, this work provides a foundation for multidisciplinary research, including multi-omics profiling, intervention trials, and longitudinal studies aimed at developing personalized, mechanism-based approaches to the diagnosis and management of ASD.

REFERENCES

1. Mallet M, Desplats V, Bouzbib C, et al. Blood ammonia in patients with chronic liver diseases: A better defined role in clinical practice. Anal Biochem. 2022; 657:114873. 10.1016/j.ab.2022.114873
2. Yokoyama S, Sugawara N, Maruo K, Yasui-Furukori N, Shimoda K. Blood Levels of Ammonia and Carnitine in Patients Treated with Valproic Acid: A Meta-analysis. Clin Psychopharmacol Neurosci. 2022;20(3):536-547. 10.9758/cpn.2022.20.3.536
3. Veltman TR, Tsai CJ, Gomez-Ospina N, Kanan MW, Chu G. Point-of-Care Analysis of Blood Ammonia with a Gas-Phase Sensor. ACS Sens. 2020;5(8):2415-2421. 10.1021/acssensors.0c00480
4. Nojima T, Naito H, Obara T, et al. Can Blood Ammonia Level, Prehospital Time, and Return of Spontaneous Circulation Predict Neurological Outcomes of Out-of-Hospital Cardiac Arrest Patients? A Nationwide, Retrospective Cohort Study. J Clin Med. 2022;11(9):2566. Published 2022 May 4. 10.3390/jcm11092566
5. Djernes L, Vilstrup H, Ott P, Eriksen PL. Blood ammonia concentration measurement - effects of sampling site and cirrhosis during induced hyperammonaemia. Metab Brain Dis. 2024;40(1):21. Published 2024 Nov 20. 10.1007/s11011-024-01442-4

6. Batallas D, Gallego JJ, Casanova-Ferrer F, et al. Blood ammonia and eye-hand coordination negatively affect health-related quality of life in women with minimal hepatic encephalopathy. Qual Life Res. 2025;34(6):1669-1682. 10.1007/s11136-025-03920-3
7. Biehl KE, Wallace ML, Cunningham M. Capillary blood is not accurate in predicting blood ammonia values using an ammonia point-of-care test in dogs. Am J Vet Res. 2025;86(4):ajvr.24.10.0324. Published 2025 Feb 3. 10.2460/ajvr.24.10.0324
8. Mercer-Smith GW, Appleton M, Hanon ÉA, Bowron A. Blood samples for ammonia analysis do not require transport to the laboratory on ice: a study of ammonia stability and cause of in vitro ammonia increase in samples from patients with hyperammonaemia. Clin Chem Lab Med. 2025;63(6):1132-1138. Published 2025 Jan 6. 10.1515/cclm-2024-1304
9. Gao Y, Zhang H, Zhong H, Yang S, Wang Q. Lactate and blood ammonia on admission as biomarkers to predict the prognosis of patients with acute mushroom poisoning and liver failure: a retrospective study. Toxicol Res (Camb). 2021;10(4):850-855. Published 2021 Jul 24. 10.1093/toxres/tfab068
10. Li L, Liu P, Li K, et al. Predictive Value of Blood Ammonia in the Prognosis of Acute Liver Failure Evaluated by Receiver Operating Characteristic Curves. Turk J Gastroenterol. 2021;32(2):164-168. 10.5152/tjg.2021.19663
11. Korman P, Kusy K, Straburzyńska-Lupa A, Kantanista A, Quintana MS, Zieliński J. Response of skin temperature, blood ammonia, and lactate during incremental exercise until exhaustion in elite athletes. Sci Rep. 2024;14(1):2237. Published 2024 Jan 26. 10.1038/s41598-024-52374-z
12. Oliveira NM, Sousa Filho LF, França TC, et al. Different dehydration levels and their impact on blood ammonia, cognitive-motor performance, and muscle damage in acclimated runners. J Sports Med Phys Fitness. 2022; 62(12):1716-1722. 10.23736/S0022-4707.22.13448-1
13. Zheng P, Qin X, Feng R, et al. Alleviative effect of melatonin on the decrease of uterine receptivity caused by blood ammonia through ROS/NF-κB pathway in dairy cow. Ecotoxicol Environ Saf. 2022; 231:113166. 10.1016/j.ecoenv.2022.113166
14. Hirota T, King BH. Autism Spectrum Disorder: A Review. JAMA. 2023;329(2):157-168. 10.1001/jama.2022.23661
15. Wang L, Wang B, Wu C, Wang J, Sun M. Autism Spectrum Disorder: Neurodevelopmental Risk Factors, Biological Mechanism, and Precision Therapy. Int J Mol Sci. 2023;24(3):1819. Published 2023 Jan 17. 10.3390/ijms24031819
16. Singhi P, Malhi P. Early Diagnosis of Autism Spectrum Disorder: What the Pediatricians Should Know. Indian J Pediatr. 2023;90(4):364-368. 10.1007/s12098-022-04363-1
17. Lamanna J, Meldolesi J. Autism Spectrum Disorder: Brain Areas Involved, Neurobiological Mechanisms, Diagnoses and Therapies. Int J Mol Sci. 2024;25(4):2423. Published 2024 Feb 19. 10.3390/ijms25042423
18. Tang SJ, Wei HL, Li CY, Huang MN. Management strategies of dental anxiety and uncooperative behaviors in children with Autism spectrum disorder. BMC Pediatr. 2023;23(1):612. Published 2023 Dec 4. 10.1186/s12887-023-04439-7
19. Botelho RM, Silva ALM, Borbely AU. The Autism Spectrum Disorder and Its Possible Origins in Pregnancy. Int J Environ Res Public Health. 2024;21(3):244. Published 2024 Feb 20. 10.3390/ijerph21030244
20. Lwanga SK, Lemeshow S: Sample size determination in health studies: A practical manual. Geneva: World Health Organization; 1991. https://lib.umku.ac.id/wp-content/uploads/2022/06/Sample-size-determination-in-health-studies-a-practical-manual.pdf
21. Sharma SK, Mudgal SK, Thakur K, Gaur R: How to calculate sample size for observational and experimental nursing research studies. Natl J Physiol Pharm Pharmacol. 2020;10(1):1–8. 10.5455/njppp.2020.10.0930717102019
22. Patra S, Kar SK. Autism spectrum disorder in India: a scoping review. Int Rev Psychiatry. 2021;33(1-2):81-112. 10.1080/09540261.2020.1761136
23. Shahane V, Kilyk A, Srinivasan SM. Effects of physical activity and exercise-based interventions in young adults with autism spectrum disorder: A systematic review. Autism. 2024;28(2):276-300. 10.1177/13623613231169058
24. Hill TL, White TC, Anthony BJ, et al. Disparities in autism spectrum disorder diagnoses among 8-year-old children in Colorado: Who are we missing?. Autism. 2021;25(1):102-113. 10.1177/1362361320950058
25. Mahajan R, Sagar R. Adequate Management of Autism Spectrum Disorder in Children in India. Indian J Pediatr. 2023;90(4):387-392. 10.1007/s12098-022-04352-4
26. Lin X, Wang G, Shen S, Zhan J. Advances in the Diagnosis and Treatment of Autism Spectrum Disorders in Children. Altern Ther Health Med. 2024;30(3):170-175. https://pubmed.ncbi.nlm.nih.gov/37883758/
27. Wilkinson S, Evans S, DeJong M. Assessing autism spectrum disorder in children with a background of maltreatment: challenges and guidance. Arch Dis Child. 2023;108(8):597-600. 10.1136/archdischild-2022-323986