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Research Article

Baseline Urinary Micronutrient, Neurotransmitter and Behavioural Profiles of Children with Autism Spectrum Disorder in Jos Metropolis, Plateau State, Nigeria

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ABSTRACT

Evidence describing urinary micronutrient and neurotransmitter profiles among children with autism spectrum disorder (ASD), particularly in sub-Saharan Africa, remains limited. This study characterised baseline urinary micronutrient and neurotransmitter profiles and parent-reported behavioural characteristics among children with ASD in Jos, Nigeria. This descriptive baseline analysis formed part of a broader three-point longitudinal repeated-measures intervention study involving 42 children with ASD recruited from three special education schools. Eight participants with complete baseline urinary micronutrient and neurotransmitter data were included in the present analysis. Spot urine samples were analysed for iron (Fe), zinc (Zn), magnesium (Mg), and selenium (Se) using Microwave Plasma-Atomic Emission Spectrometry for glutamate and γ-aminobutyric acid (GABA) using High-Performance Liquid Chromatography with fluorescence detection. Urinary concentrations were not creatinine-normalised. Data were summarised using median and interquartile range. Urinary Fe, Zn and Se concentrations were below the laboratory reference ranges in all eight participants, whereas Mg concentrations remained within range. Median (IQR) concentrations were 1.255 (1.089-1.739) ppm for Fe, 0.528 (0.365-0.672) ppm for Zn, 5.818 (5.290-6.116) ppm for Mg and 1.157 (1.114-1.209) ppm for Se. Median urinary glutamate and GABA concentrations were 16.358 (14.746-18.759) and 0.421 (0.364-0.608) µmol/L, respectively, with a median GABA: glutamate ratio of 0.026 (0.024-0.041). Behavioural scores varied across participants. The study provides descriptive baseline urinary biochemical and behavioural data from an under-represented Nigerian ASD population. Urinary micronutrient findings represent excretory profiles rather than definitive measures of systemic nutritional status, and the uncorrected spot-urine measurements warrant cautious interpretation. Larger studies incorporating creatinine-normalised urinary measures and established systemic nutritional biomarkers are needed.

Keywords: *Autism; Urinary micronutrients; Urinary neurotransmitters; γ-aminobutyric acid; (GABA): Glutamate ratio*

INTRODUCTION

Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder characterised by persistent deficits in social communication and social interaction, accompanied by restricted, repetitive patterns of behaviour, interests, or activities (American Psychiatric Association 2022). The prevalence of ASD has increased steadily over the past two decades, with recent estimates indicating that approximately one in every 31 children is diagnosed with the condition in the United States, while similar upward trends have been reported globally (Centers for Disease Control and

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Prevention CDC, 2025; World Health Organization WHO, 2023). Although improvements in awareness, diagnostic criteria, and access to screening services have contributed to this increase, the biological mechanisms underlying ASD remain incompletely understood. Rather than arising from a single aetiological pathway, ASD is now recognised as a multifactorial disorder resulting from complex interactions among genetic susceptibility, environmental exposures, immune dysregulation, metabolic disturbances, oxidative stress, and nutritional factors. This biological complexity contributes to the marked heterogeneity observed in clinical presentation and has prompted increasing interest in identifying measurable biochemical markers that may improve understanding of the disorder and inform targeted therapeutic interventions.

Among the biological factors implicated in ASD, micronutrient status has received considerable attention because of its fundamental role in brain development, neurotransmitter synthesis, synaptic plasticity, and antioxidant defence. Iron, zinc, magnesium, and selenium are particularly important for normal neurodevelopment, serving as essential cofactors in enzymatic reactions involved in neuronal differentiation, myelination, neurotransmission, and protection against oxidative stress (Beard, 2008; Prasad, 2013; Rayman, 2012). Deficiencies of these micronutrients during critical periods of development have been associated with impaired cognitive function, altered neuronal connectivity, and behavioural abnormalities, raising the possibility that nutritional inadequacies may contribute to the pathophysiology or clinical manifestations of ASD. Several studies have reported lower concentrations of one or more of these micronutrients among children with ASD compared with neurotypical controls (Marinov *et al*., 2024; Saghazadeh *et al*., 2017). However, findings remain inconsistent across populations, partly because of differences in study design, dietary habits, biological specimens analysed, laboratory methodologies, and geographical variation. Consequently, the extent to which micronutrient deficiencies contribute to the biochemical alterations observed in ASD remains incompletely understood, highlighting the need for further investigation in diverse populations.

Biological assessment of micronutrients may involve different biological matrices, depending on the micronutrient and the purpose of measurement. While blood-based biomarkers are commonly used to assess systemic micronutrient status, urinary measurements can provide information on the excretion and handling of micronutrients and may therefore contribute complementary information on their biochemical profiles. However, urinary concentrations are influenced by factors such as recent intake, renal handling and urine dilution and should not be interpreted as direct measures of tissue stores or systemic nutritional status. The choice of urine as the biological specimen was particularly important as the broader study was longitudinal and involved children with ASD, requiring repeated sample collection at multiple assessment points. Urine was selected because it provides a non-invasive and relatively practical specimen for repeated collection in this population, while still providing relevant biochemical information. Thus, the use of urine in the present study was not intended to replace blood-based assessment of systemic micronutrient status, but rather to characterise urinary micronutrient profiles.

The regulation of excitatory and inhibitory neurotransmission is fundamental to normal brain development and function, with glutamate and gamma-aminobutyric acid (GABA) serving as the principal excitatory and inhibitory neurotransmitters, respectively. Together, these neurotransmitters maintain the excitatory-inhibitory (E/I) balance required for neuronal excitability, synaptic plasticity, sensory processing, learning, and behaviour (Cellot & Cherubini, 2014). Increasing evidence suggests that disruption of this balance may contribute to the pathophysiology of ASD, giving rise to the widely accepted excitatory-inhibitory imbalance hypothesis (Rubenstein & Merzenich, 2003). Studies employing neuroimaging, post-mortem brain tissue, genetic analyses, and cerebrospinal fluid biomarkers have reported alterations in glutamatergic and GABAergic signalling pathways among individuals with ASD (Fatemi *et al*., 2009; Uzunova *et al*., 2016). Although peripheral measurements of urinary GABA and glutamate cannot be assumed to directly reflect central nervous system neurotransmission, they may provide useful, non-invasive insights into systemic biochemical processes associated with neurodevelopmental disorders. Consequently, characterising urinary concentrations of these neurotransmitters and describing their relative relationship using the GABA:glutamate ratio may contribute to a broader understanding of neurochemical patterns in children with ASD, while recognising that universally validated urinary reference ranges and diagnostic cut-off values for these biomarkers have not yet been established.

Despite growing evidence linking micronutrient status and neurotransmitter dysregulation to ASD, important knowledge gaps remain. Much of the existing literature has investigated micronutrient deficiencies or neurotransmitter abnormalities independently, with relatively few studies examining both nutritional status and neurotransmitter profiles within the same cohort of children with ASD. Furthermore, while alterations in GABAergic and glutamatergic signalling have been demonstrated using neuroimaging, cerebrospinal fluid, plasma, and post-mortem brain tissue, comparatively little attention has been given to the characterisation of urinary GABA and glutamate as non-invasive biochemical markers. The absence of universally accepted reference intervals for urinary neurotransmitters further limits the clinical interpretation of these biomarkers, underscoring the need for descriptive studies that establish baseline biochemical profiles rather than diagnostic thresholds. In addition, most available evidence has been generated in high-income countries, with comparatively limited data from sub-Saharan Africa, where differences in dietary patterns, environmental exposures, healthcare access, and micronutrient availability may influence biochemical and neurodevelopmental outcomes. Addressing these gaps is essential to improving understanding of the nutritional and

neurochemical characteristics of children with ASD across diverse populations.

Against this background, an important question arises: could widespread micronutrient deficiencies contribute to alterations in the urinary GABA-glutamate neurotransmitter profile among children with autism spectrum disorder? Although the present study was not designed to establish causal relationships, characterising both urinary micronutrient profiles and the urinary GABA-glutamate neurotransmitter profile within the same cohort provides an opportunity to explore their coexistence at baseline before nutritional intervention. Given the critical roles of iron, zinc, magnesium, and selenium in neurotransmitter synthesis, synaptic function, oxidative defence, and neuronal signalling, evaluating these biochemical parameters concurrently may provide insights into the nutritional and neurochemical landscape of children with ASD. Furthermore, describing the GABA:glutamate ratio as a measure of the relative relationship between these neurotransmitters, rather than as a diagnostic indicator, offers an additional perspective for interpreting urinary neurotransmitter profiles in the absence of universally accepted reference standards. Such baseline characterisation is essential for informing future intervention studies aimed at determining whether micronutrient supplementation may be accompanied by changes in neurotransmitter homeostasis and clinical outcomes.

In view of the existing knowledge gaps, the present study aimed to characterise baseline urinary micronutrient profiles and the urinary GABA-glutamate neurotransmitter profile among children with autism spectrum disorder prior to micronutrient intervention. Specifically, the study characterised urinary iron, zinc, selenium and magnesium concentrations in relation to laboratory reference ranges used by the analytical laboratory, characterised urinary concentrations of GABA and glutamate, and described the GABA-glutamate ratio as a measure of the relationship between these neurotransmitters. It was anticipated that the findings would establish a comprehensive biochemical baseline for evaluating the effects of subsequent micronutrient intervention on neurochemical and behavioural outcomes.

MATERIALS AND METHODS

Study design

This study reports the baseline findings from a quantitative longitudinal one-group intervention study designed to investigate the effects of micronutrient supplementation on selected biochemical and behavioural parameters in children with Autism Spectrum Disorder (ASD). The present paper is based exclusively on data collected before the commencement of the intervention and therefore adopts a descriptive cross-sectional approach to characterise the participants' baseline urinary micronutrient status and urinary GABA-glutamate neurotransmitter profile. The baseline assessment was undertaken to establish the nutritional and neurochemical characteristics of the study cohort prior to micronutrient supplementation and to provide a reference for evaluating subsequent intervention-related changes.

Study location

The study was conducted among children with autism spectrum disorder (ASD) enrolled in three special education schools within Jos Metropolis, Plateau State, Nigeria: Otana Integrated School, Ganaka Memorial International School, and Open Doors Special Education Centre. These schools provide specialised educational and supportive services for children with developmental and learning disabilities, including ASD, and served as the participant recruitment sites.

Laboratory analyses were conducted at the Department of Biochemistry, University of Jos, Jos, Nigeria, and the Multi-User Science Research Laboratory, Ahmadu Bello University, Zaria, Nigeria. Initial sample handling, preservation, and preparation were undertaken at the University of Jos, while quantitative analyses of urinary micronutrients and neurotransmitters were performed using advanced analytical instrumentation at the Multi-User Science Research Laboratory under standardised operating procedures and quality control protocols.

Study population

The study population comprised children, ages 4-15 years, diagnosed with Autism Spectrum Disorder (ASD) who were enrolled in selected special education schools within Jos Metropolis, Plateau State, Nigeria. Eligible participants had a confirmed diagnosis of ASD documented in their medical records prior to enrolment into the study. The study focused on characterising the baseline urinary micronutrient status and urinary GABA-glutamate neurotransmitter profile of this cohort before the commencement of micronutrient intervention.

Inclusion and exclusion criteria

Children were eligible for inclusion in the study if they had a clinically confirmed diagnosis of Autism Spectrum Disorder (ASD), were enrolled in one of the selected special education schools within Jos Metropolis, and whose parents or legal guardians provided written informed consent for participation. Participants were also required to meet the study requirements for urine sample collection and behavioural assessment at baseline prior to the commencement of micronutrient intervention.

Children were excluded if they had severe medical conditions that could interfere with study participation, were receiving medications or nutritional supplements considered likely to influence micronutrient status or neurotransmitter metabolism during the baseline assessment period, or if adequate urine samples could not be obtained for laboratory analysis. Participants with incomplete baseline biochemical or behavioural data were also excluded from the present analysis.

Ethical clearance

Ethical approval was obtained from the University of Jos Central Health Research Ethics Committee (UJCHREC) before the commencement of the research, with approval number UJCHREC/25/011. Permission was also sought from the management of participating schools. Written informed consent was obtained from parents/guardians with assent from the adolescent child, where necessary. All research procedures employed non-invasive methods for sample collection, using urine samples and behavioural assessments were carried out using the parents' reported behaviour questionnaire. No invasive clinical procedures were performed. Confidentiality of participant information was ensured by assigning unique identification codes, and all data were used strictly for research purposes. Participation was entirely voluntary, and consent was permitted to be withdrawn from the study at any stage without penalty.

Sample size and sample technique

Participants were recruited using a purposive sampling technique from three selected special education schools within Jos Metropolis, Plateau State, Nigeria. A total of 42 children with clinical confirmed autism spectrum disorder were initially identified in the three special schools. Parents/guardians of 10 children refused giving consent, 32 gave consents. All 32 participants commenced the intervention study.

During the longitudinal follow up, 24 participants discontinued the study. These included 4 participants who relocated, 12 whose parent/guardian later withdrew consent and 8 who were disqualified because of non-compliance with the study procedure. Only 8 participants completed the actual longitudinal intervention study, consequently, this manuscript presents the baseline data of the 8 completers of the study.

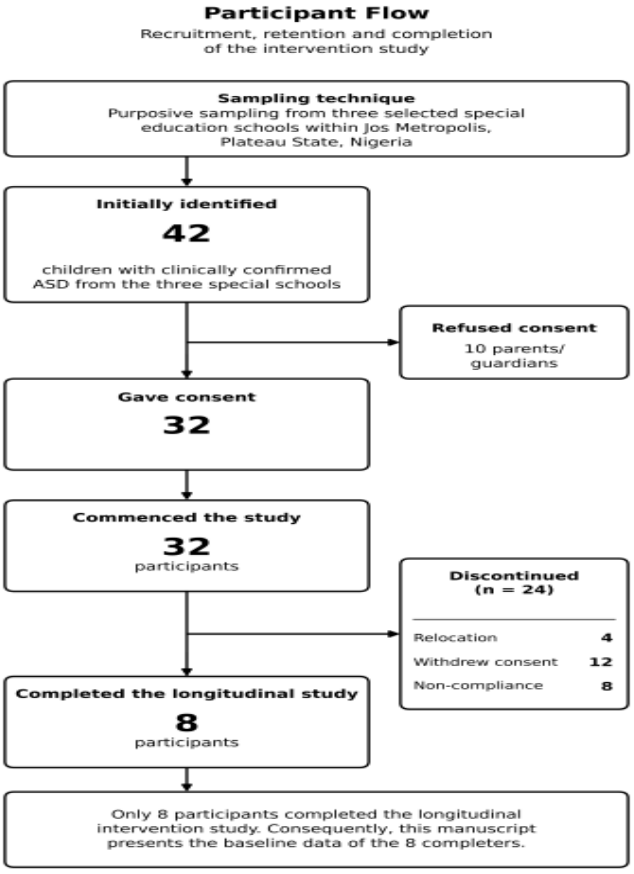


Figure 1: Participants Recruitment Flowchart

Data collection

Baseline data were collected before the commencement of micronutrient intervention and included behavioural assessment, urine sample collection, laboratory determination of urinary micronutrients and neurotransmitters. All assessments were performed according to standardised procedures to ensure consistency and reliability of the data collected.

Behavioural assessment

Behavioural characteristics of the participants were assessed at baseline using a researcher-developed parents' reported questionnaire. The questionnaire comprised ten (10) domains namely, attention/focus, communication, social interaction, emotional balance, sleep pattern, appetite, energy/activity, learning/memory, behaviour regulation and general health. Each domain was rated on a 5-point Likert scale ranging from 1 (not at all) to 5 (very often), giving a possible total score of 10-50, with higher scores indicating more favourable parent-observed behavioural characteristics. Parents reported the child's usual behaviour over the preceding two weeks. The instrument underwent content review by independent professionals with expertise in Special Needs Education and Biochemistry to ensure that the questionnaire items were relevant, appropriate, and representative of the behavioural domains assessed. Formal psychometric reliability testing was not performed therefore, the behavioural findings should be interpreted as exploratory parent-reported observations.

Urine sample collection

First-morning midstream urine samples were collected from each participant at baseline before the commencement of micronutrient intervention. Samples were obtained using sterile universal containers under appropriate hygienic conditions and were labelled with unique participant identification codes to maintain confidentiality. Immediately after collection, the urine samples were preserved and transported under recommended conditions to the Department of Biochemistry, University of Jos, for preliminary processing and storage before laboratory analysis at the Multi-User Science Research Laboratory, Ahmadu Bello University, Zaria. Standard procedures for sample handling and preservation were followed throughout the collection, transportation, and storage processes to minimise sample degradation and maintain analytical integrity.

Urinary creatinine was not measured in the present study, consequently, the urinary micronutrient and neurotransmitter concentrations were reported as unadjusted concentrations and were not normalized to urinary creatinine.

Laboratory determination of urinary micronutrients

Urinary concentrations of iron (Fe), zinc (Zn), magnesium (Mg), and selenium (Se) were determined using Microwave Plasma-Atomic Emission Spectrometry MP-AES (Agilent 4200 MP-AES, Agilent Technologies, Melbourne. Australia) at the Multi-User Science Research Laboratory, Ahmadu Bello University, Zaria, Nigeria. First-morning midstream urine samples were collected in sterile urine containers, labelled with unique participant identification codes, and transported under cold-chain conditions. Sample preparation was initiated at the Department of Biochemistry, University of Jos, before transfer to the analytical laboratory for elemental analysis.

Trace metal quantification was performed using validated MP-AES analytical procedures. The technique is based on the excitation of atoms within a microwave-induced nitrogen plasma, where nebulised sample aerosols are atomised and excited to higher energy states. As the excited atoms return to their ground states, they emit light at characteristic wavelengths, with emission intensity directly proportional to the concentration of each element present in the sample. The use of nitrogen plasma generated from atmospheric air provides high analytical sensitivity while reducing operational costs and eliminating the need for combustible gases. Standard operating procedures, instrument calibration, and internal quality control measures were implemented throughout the analytical process to ensure the accuracy, precision, and reproducibility of the results. Urinary micronutrient concentrations were interpreted using the laboratory reference ranges provided by the analytical laboratory (The Multi-User Science Research Laboratory, Ahmadu Bello University, Zaria).

Urinary neurotransmitter analysis

Urinary concentrations of γ-aminobutyric acid (GABA) and glutamate were determined from baseline urine samples using high-performance liquid chromatography coupled with fluorescence detection HPLC-FLD (Agilent Infinity HPLC system, Agilent Technologies 1260 Infinity, Santa Clara, CA, USA) at the Multi-User Science Research Laboratory, Ahmadu Bello University, Zaria, Nigeria. Urine samples were collected as first-morning specimens and immediately treated with hydrochloric acid (HCl) and ascorbic acid according to standard laboratory procedures to preserve the stability of the amino acid neurotransmitters and minimise oxidative degradation during storage and transportation. The preserved samples were centrifuged to remove particulate matter, and the resulting supernatant was used for chromatographic analysis.

Prior to analysis, urine samples were subjected to pre-column derivatisation using o-phthalaldehyde (OPA) reagent in the presence of 3-mercaptopropionic acid to convert the amino acid neurotransmitters into highly fluorescent isoindole derivatives suitable for sensitive detection. Chromatographic separation of GABA and glutamate was achieved on a reversed-phase C18 column using a phosphate buffer-acetonitrile mobile phase under controlled analytical conditions. Quantification of the neurotransmitters was performed by comparing the chromatographic peak areas of the derivatised samples with those of known calibration standards. Urinary concentrations of GABA and glutamate were expressed in micromoles per litre (µmol/L) and used for subsequent baseline analyses.

Calculation of the GABA: Glutamate ratio

The urinary GABA: glutamate ratio was calculated for each participant at baseline by dividing the urinary concentration of γ-aminobutyric acid (GABA) by the corresponding urinary concentration of glutamate. The ratio was computed using the quantified neurotransmitter concentrations (µmol/L) obtained from HPLC analysis and was used as a descriptive index of the relative balance between inhibitory (GABA) and excitatory (glutamate) neurotransmitter levels in the study population.

RESULTS

Baseline characteristics of the study participants

A total of eight children with autism spectrum disorder (ASD) who had complete baseline urinary micronutrient and neurotransmitter data from the broader three-point longitudinal repeated-measures intervention study were included in the present baseline analysis. The median age of the participants was 6.5 years (IQR: 5.0-9.0 years), with ages ranging from 4 to 14 years.

The participants were predominantly males (87.5%), with one female participant (12.5%). The median body weight was 24.5 kg (IQR: 20.5-29.0 kg), while the median height was 119.0 cm (IQR: 107.0-129.0 cm). Participants were recruited from three special educational centres, namely Otana Integrated School (37.5%), Open Doors Special Education Centre (37.5%), and Ganaka Memorial International School (25.0%) all within Jos metropolis, Plateau State Nigeria (Table 1).

Table 1: Baseline Characteristics of the Study Participants (n = 8)

Variables	Values
Number of participants, n	8
Age (years), Median (IQR)	6.5 (5.0-9.0)
Age range (years)	4-14
Sex, n (%)	
Male	7 (87.5)
Female	1 (12.5)
Weight (kg), Median (IQR)	24.5 (20.5-29.0)
Weight range (kg)	15-70
Height (cm), Median (IQR)	119.0 (107.0-129.0)
Height range (cm)	94-160
Recruitment site, n (%)	
Otana Integrated School	3 (37.5)
Open Doors Special Education Centre	3 (37.5)
Ganaka Memorial International School	2 (25.0)

Baseline urinary micronutrient concentrations

The baseline urinary concentrations of the selected micronutrients among the participating children with autism spectrum disorder (ASD) are presented in Table 2. The reported urinary concentrations were not adjusted for creatinine, as urinary creatinine was not measured. The median (IQR) urinary concentrations of iron, zinc, magnesium and selenium were 1.255 (1.089-1.739) ppm, 0.528 (0.365-0.672) ppm, 5.818 (5.290-6.116) ppm, and 1.157 (1.114-1.209) ppm, respectively. Individual participant values demonstrated variability across all four micronutrients.

Urinary iron concentrations ranged from 0.758 to 2.103 ppm, zinc concentrations ranged from 0.253 to 1.051 ppm, magnesium concentrations ranged from 3.584 to 8.129 ppm, while selenium concentrations ranged from 1.100 to 1.375 ppm.

Table 2: Baseline Urinary Micronutrient Concentrations among Children with Autism Spectrum Disorder (ASD) (N = 8)

Participant	Fe (ppm)	Zn (ppm)	Mg (ppm)	Se (ppm)
RJ	0.862	0.934	0.757	0.229
SC	0.701	1.171	0.565	0.896
TS	0.645	1.158	1.26	0.844
TR	0.974	1.097	1.009	0.406
EB	0.556	0.873	1.02	0.613
AB	0.79	1.282	1.288	0.291
OC	1.223	0.761	0.55	0.295
GF	1.003	1.888	1.399	0.572
Median (IQR)	1.255(1.089-1.739)	0.528(0.365-0.672)	5.818(5.290-6.116)	1.157(1.114-1.209)

Participants' identities were coded as RJ, SC, TS, TR, etc. Fe = iron; Zn = zinc; Mg = magnesium; Se = selenium. Values represent unadjusted urinary concentrations as urinary creatinine was not measured. They are expressed as urinary concentrations in ppm. Comparisons with reference limits represent the laboratory reference ranges used for the interpretation of urinary concentrations in this study and should not be interpreted as direct measures of systemic micronutrient status

Baseline urinary neurotransmitter concentrations and GABA: Glutamate ratio

The baseline urinary concentrations of glutamate, GABA and the calculated GABA: glutamate ratio among the participating children with Autism spectrum disorder (ASD) are presented in Table 3. The median (IQR) urinary glutamate concentration was 16.358 (14.746-18.759) µmol/L, while the median (IQR) urinary GABA concentration was 0.421 (0.364-0.608) µmol/L. The median (IQR) GABA: glutamate ratio was 0.026 (0.024-0.041). Individual participant values demonstrated variability in the urinary concentrations of both neurotransmitters. Urinary glutamate concentrations ranged from 9.499 to 27.375 µmol/L, whereas urinary GABA concentrations ranged from 0.315 to 0.839 µmol/L. Similarly, the calculated GABA: glutamate ratio ranged from 0.012 to 0.048 across the study participants. Although the GABA: glutamate ratio was consistently low across all participants, modest inter-individual variability was observed, reflecting differences in the relative urinary concentrations of GABA and glutamate at baseline

Table 3: Baseline Urinary Neurotransmitter Concentrations and GABA: Glutamate Ratio among Children with Autism Spectrum Disorder (ASD) (n = 8)

Participant	Glutamate (µmol/L)	GABA (µmol/L)	GABA: Glutamate Ratio
RJ	22.308	0.593	0.026
SC	16.395	0.412	0.025
TS	16.320	0.429	0.026
TR	17.576	0.839	0.048
EB	27.375	0.335	0.012
AB	9.499	0.374	0.039
OC	14.387	0.651	0.045
GF	14.866	0.315	0.021
Median (IQR)	16.358(14.746-18.759)	0.421(0.364-0.608)	0.026 (0.024-0.041)

GABA = γ-aminobutyric acid. The GABA:glutamate ratio was calculated by dividing urinary GABA concentration by the corresponding urinary glutamate concentration.

Baseline behavioural scores

Baseline behavioural scores obtained using the researcher-developed Parents' Reported questionnaire are presented in Table 4. The questionnaire assessed 10 autism-related behavioural and functional domains: attention and focus, communication, social interaction, emotional balance, sleep

pattern, appetite and eating, energy and activity, learning and memory, behaviour regulation, and general health. Parents rated their child's usual behaviour and daily functioning over the preceding two weeks using a 5-point Likert scale ranging from 1 (not at all) to 5 (very often). The resulting total score ranged from 10 to 50, with higher scores indicating more frequent endorsement of the behavioural or functional characteristics assessed. The participants had a median (IQR) behavioural score of 28.5 (26.5-31.0), with individual scores ranging from 24 to 36. The variation in scores indicates differences in parents' reported behavioural and functions characteristics across the participants at baseline.

Table 4: Baseline Behavioural Scores of Children with Autism Spectrum Disorder (ASD) Assessed using the Parents' Reported Questionnaire (n = 8).

Participant	Behavioural Score
RJ	36
SC	31
TS	24
TR	27
EB	31
AB	26
OC	30
GF	27
Median (IQR)	28.5 (26.5-31.0)

Scores represents the summed responses across the 10 questionnaire domains: IQR=Interquartile range

DISCUSSION

The present study characterised the baseline urinary micronutrient profile, urinary neurotransmitter profile, GABA:glutamate ratio and behavioural characteristics of children with Autism Spectrum Disorder (ASD) attending special schools in Jos Metropolis, Plateau State, Nigeria. Iron, zinc and selenium concentrations were below the lower limits of the laboratory reference ranges used in the study in all eight participants, whereas urinary magnesium concentrations remained within the reference range. Urinary glutamate and GABA concentrations varied between participants, with consistently low GABA:glutamate ratios, while behavioural scores also showed inter-individual variation. These findings provide a baseline biochemical and behavioural profile of the study cohort.

Baseline urinary micronutrient profile

The urinary micronutrient profile was characterised by relatively low iron, zinc and selenium concentrations and urinary magnesium concentrations within the laboratory reference range. This pattern is of interest in the context of the nutritional vulnerability reported among children with ASD. Feeding difficulties and food selectivity are common in this population and may restrict dietary variety and nutrient intake (Sharp *et al*., 2013). Recent evidence likewise indicates differences in dietary and micronutrient characteristics among children with ASD, although findings vary across populations and assessment methods (Alhrbi *et al*., 2025).

Iron is essential for mitochondrial energy metabolism, myelination and neurotransmitter synthesis, making its low urinary concentration in the present cohort biologically noteworthy. However, evidence regarding iron status in ASD remains heterogeneous; a systematic review and meta-analysis found no significant overall difference in serum ferritin or hair iron between children with ASD and controls, despite reported associations with iron deficiency in some studies (Tseng *et al*., 2018). The present finding therefore provides information on urinary iron excretion rather than establishing depleted systemic iron stores.

Similarly, zinc plays important roles in neuronal development, synaptic function and neurotransmission. The uniformly low urinary zinc concentrations observed in this cohort are therefore relevant to the overall biochemical profile. However, zinc findings in ASD differ according to the biological matrix examined. A systematic review identified considerable heterogeneity among studies, while a recent meta-analysis found an association between ASD and lower blood zinc concentrations but not urinary zinc concentrations (doNascimento *et al*., 2023; Liu *et al*., 2025). The present urinary finding should consequently be interpreted within the context of the selected biological matrix.

Urinary magnesium concentrations remained within the laboratory reference range in all participants. Magnesium contributes to neuronal excitability, calcium homeostasis and NMDA receptor regulation, and its observed profile provides a useful contrast to the lower urinary concentrations of iron, zinc and selenium. The finding does not establish systemic magnesium adequacy but indicates that no abnormal urinary magnesium profile was identified using the laboratory reference range.

Selenium concentrations were also below the lower laboratory reference limit in all participants. Selenium is required for selenoproteins involved in antioxidant defence and cellular redox regulation, processes of interest in ASD research given the evidence linking oxidative stress with the disorder (Bjørklund *et al*., 2020). The concurrent urinary profiles of iron, zinc and selenium are therefore noteworthy, though the present study cannot determine whether they arose from dietary intake, absorption, renal handling or other physiological factors.

Overall, the findings indicate a distinct urinary micronutrient profile within this cohort, with low urinary iron, zinc and selenium concentrations alongside urinary magnesium concentrations within the laboratory reference range. These observations provide useful baseline biochemical information and support further investigation using complementary dietary and systemic nutritional measures, particularly in longitudinal studies examining the response to micronutrient intervention.

Baseline urinary neurotransmitter profile and GABA: Glutamate ratio

The present study demonstrated measurable urinary glutamate and γ-aminobutyric acid (GABA) concentrations with inter-individual variation across the cohort. The median urinary glutamate concentration was 16.357 µmol/L and that of GABA was 0.421 µmol/L. The GABA:glutamate ratio was consistently low across participants, although no universally

established reference interval or clinical threshold currently exists for urinary GABA, glutamate or their ratio. These findings are therefore best interpreted as a descriptive peripheral neurochemical profile rather than evidence of neurotransmitter abnormality.

Glutamate and GABA are the principal excitatory and inhibitory neurotransmitters, respectively, and their interaction is central to neuronal signalling and the proposed excitatory-inhibitory (E/I) mechanisms implicated in ASD. Evidence from central measures, including magnetic resonance spectroscopy, has reported alterations in glutamatergic and GABAergic measures in some children with ASD, although findings are not uniform across brain regions and populations (Johnson *et al*., 2023). Such evidence provides biological context for investigating these neurotransmitters but cannot be directly extrapolated to urinary measurements.

Evidence specifically concerning urinary neurotransmitters in ASD remains limited. Gevi *et al*. (2020) identified alterations in urinary metabolites related to neurotransmitter metabolism among children with ASD, including reduced urinary GABA, supporting the potential value of urinary metabolic profiling for characterising peripheral biochemical alterations. The present findings add baseline quantitative data on urinary glutamate and GABA from a Nigerian cohort.

The low GABA:glutamate ratios observed should consequently be regarded as a descriptive feature of this cohort rather than a marker of central E/I imbalance or a diagnostic indicator. Further studies incorporating creatinine-normalised measurements, appropriate comparison groups and complementary systemic or central biomarkers are required to determine the biological and clinical significance of these urinary profiles.

Behavioural characteristics

The behavioural assessment revealed appreciable inter-individual variation, with scores ranging from 24 to 36 and a median of 28.95 (IQR 26.5-31.0). This variability is consistent with the recognised clinical heterogeneity of ASD, in which children may differ considerably in social communication, emotional regulation, adaptive functioning and behavioural characteristics (Lord *et al.*, 2020; Zeidan *et al*., 2022). The broad range of domains assessed in the present study, including communication, social interaction, attention, emotional balance, sleep, eating, learning, behaviour regulation and general health, therefore provides a useful description of the diverse behavioural characteristics observed within this cohort.

The use of parent-reported observations is particularly relevant because many behaviours assessed in ASD are expressed across everyday settings and may not be fully captured during clinical or structured assessments. Parent-reported measures are commonly used to assess adaptive and functional characteristics in autistic children, although ratings may vary according to the setting and informant. Thus, the variation observed in the present study may represent genuine differences in the children's day-to-day functioning, while also reflecting the context in which behaviours were observed.

The behavioural findings are also noteworthy when considered alongside the heterogeneous urinary micronutrient and neurotransmitter profiles observed in the same participants. Although the present descriptive design does not permit attribution of behavioural differences to micronutrient or neurotransmitter status, the coexistence of behavioural and biochemical variability supports the multifactorial nature of ASD and provides a basis for further investigation. Larger longitudinal studies using established behavioural instruments alongside dietary, systemic nutritional and peripheral biochemical measures may help clarify whether changes in nutritional and neurochemical profiles correspond with changes in everyday behavioural functioning.

CONCLUSION

This study provides an integrated baseline characterisation of urinary micronutrient and peripheral neurotransmitter profiles alongside parent-reported behavioural characteristics among children with ASD in Jos, Nigeria. The observed urinary profiles showed low Fe, Zn and Se values relative to the laboratory reference ranges, while Mg remained within range, alongside variable urinary glutamate and GABA concentrations and consistently low GABA: glutamate ratios. Behavioural scores also varied across participants, reflecting the heterogeneous characteristics of ASD. These findings provide valuable descriptive data from an under-represented Nigerian population and demonstrate the feasibility of combining non-invasive biochemical and behavioural assessments in this population. Importantly, urinary findings should be interpreted as excretory profiles rather than definitive measures of systemic nutritional status or central neurotransmission. The study therefore provides a useful foundation for larger, adequately powered longitudinal investigations incorporating systemic nutritional biomarkers, creatinine-normalised urinary measurements and standardised behavioural assessments.

Strengths and Limitation

A major strength of this study is its integrated characterisation of urinary micronutrient profiles, urinary glutamate and GABA, the GABA: glutamate ratio, and parent-reported behavioural characteristics within the same cohort of children with ASD. The study also provides data from a relatively under-represented Nigerian population and employed established analytical techniques, including MP-AES for micronutrients and HPLC-FLD for neurotransmitter quantification. The non-invasive urine-based approach further enabled the collection of biochemical data from children undergoing repeated assessments within the broader longitudinal study.

The findings should, however, be interpreted in light of the small number of participants with complete biochemical data and the resulting potential for selection bias, as well as the absence of a neurotypical control group. Urinary

creatinine was not measured, therefore spot-urine concentrations could not be normalised for urinary dilution. This may have contributed to inter-individual variation in measured urinary concentrations. Urinary micronutrient concentrations therefore represent excretory profiles rather than definitive measures of systemic nutritional status. In addition, the researcher-developed behavioural instrument underwent expert content review but no formal psychometric evaluation beyond this review.

Institutional Review Board

Ethical approval was obtained from the University of Jos Central Health Research Ethics Committee (UJCHREC) with approval number UJCHREC/25/011.

Data Availability

Not applicable.

AUTHORS' CONTRIBUTIONS

Conceptualization, GCO. and JII.; methodology, GCO. JII. and MDS.; validation, MDS. and JII.; formal analysis, GCO. and YMU.; investigation, GCO.; resources, GCO. and JII.; data curation, GCO., YMU and MDS.; writing-original draft preparation, GCO.; writing-review and editing, GCO., YMU., JII. and MDS; supervision, MDS., JII. and YMU.; project administration, GCO.; funding acquisition, GCO.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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