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RESEARCH

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Cellulose acetate electrophoretic separation of serum and urine proteins in Nigerian children with autism spectrum disorders

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Abstract

Background Autism spectrum disorder (ASD) is a neurodevelopmental disorder (NDD) characterized by social communication challenges and restricted, repetitive behaviors. While genetic and environmental factors are known to contribute to ASD, the role of the immune system remains unclear. This study investigated the separation patterns of serum and urine proteins in Nigerian children with ASD compared to typically developing children and children with other NDDs.

Methods Forty-seven participants aged 3–8 years were recruited, including 16 children diagnosed with ASD and 16 children with other NDDs, both according to DSM-5 criteria, along with 15 neurotypical children. Blood and urine samples were collected for protein analysis. Total protein and albumin levels were measured in both serum and urine using established methods. Protein separation in serum and urine was performed using cellulose acetate electrophoresis, followed by densitometry analysis of the electrophoretic patterns.

Results The results revealed no significant differences in total serum protein levels and most protein fractions between the groups. However, children with other NDDs exhibited significantly lower levels of alpha-2 globulin compared to neurotypical children. Conversely, both ASD and NDD groups showed significantly higher gamma globulin levels compared to the control group. Interestingly, spot urine protein levels were significantly higher in children with ASD compared to neurotypical children.

Conclusion The observed changes in alpha-2 and gamma globulin levels suggest potential immune system involvement in ASD and other NDDs. The higher urine protein excretion in the ASD group warrants further investigation to explore the potential of urinary protein biomarkers for ASD diagnosis.

Keywords Serum proteins, Cellulose acetate electrophoresis, Autism spectrum disorder, Urine protein

Background

Autism Spectrum Disorder (ASD) is one of most common NDDs, affecting 1 in 100 children worldwide [1]. It manifests with core challenges in social interaction and communication, alongside repetitive behaviors. While both genetic and environmental factors are believed to play a role in the development of ASD, the exact mechanisms remain unclear [2]. Recent research suggest that immune dysfunction might be a significant contributor to ASD. This dysfunction could be linked to genetic variations, such as those in HLA-B, LILRB2, and HLA-DQB1

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genes [3]. These genetic susceptibilities are thought to interact with environmental factors like exposure to toxins, triggering atypical immune reactions that ultimately impact neuronal development [4]. Findings of immune irregularities in autistic children include the observations of reduced cytotoxic effect of Natural Killer (NK) cells [5, 6], altered monocyte responses to Toll-Like Receptor (TLR) stimulation [7], decreased T cell population and an altered ratio of T helper to T suppressor cells [8], and hypergammaglobulinemia [9, 10].

Among the various immune system abnormalities associated with ASD, altered immunoglobulin (Ig) levels are frequently reported. However, existing studies lack consensus on the specific changes in these proteins, particularly their subtypes [4]. Specifically, IgG and IgM, constituting approximately 85% of serum immunoglobulins, were found be increased [9, 10] or reduced [11, 12] in autistic children compared to neurotypical children. These differences could be attributed to variations in study populations including age and analytical methods employed [13]. This highlights the need for further investigation using different populations and techniques. Serum protein electrophoresis is a simple, reliable technique utilized for separating serum proteins. Multiple investigations have highlighted its significance in diagnosing various diseases or disorders, including chronic infection, nephrotic syndrome, and multiple myeloma [14]. However, its application in uncovering altered immunoglobulin levels associated with ASD, along with accompanying changes in the overall serum and urine protein profile, remains limited.

A study by Croonenberghs, Wauters [15] explored the potential of serum protein electrophoresis in a Caucasian population, revealing distinct protein separation patterns in autistic children compared to controls. They found elevated total protein, albumin, and gamma globulin (particularly IgG) levels. Conversely, ElBaz, Zaki [16] investigated protein electrophoresis in Egyptian autistic children, observing no significant differences in protein levels between autistic and healthy children. These contrasting findings, even within a similar analytical method, highlight the need for further research in diverse populations. Hence, the current research aims to explore both serum and urine protein profiles in sub-Sahara African population using cellulose acetate electrophoresis. This simple approach can provide valuable initial insights into potential immunological alterations associated with ASD, particularly in resource-limited settings.

Methods

Sample collection

This research is a case–control study comprising 47 participants in age range 3 to 8 years, including 16 children with ASD as cases, 16 children with related NDDs as positive controls and 15 neurotypical children as negative controls. We included children with other NDDs (comprising of children diagnosed with attention-deficit/hyperactivity disorder (ADHD), intellectual disability, conduct disorders, and cerebral palsy) alongside children with ASD and typically developing children to explore whether the observed protein patterns are specific to ASD or common across a spectrum of NDDs. This comparison allows for a more nuanced understanding of the potential immune system involvement in ASD and the identification of any unique protein markers that could aid in distinguishing ASD from other NDDs. Both cases and positive controls were recruited from the Child and Adult Psychiatry clinic of University College Hospital (UCH), Ibadan, following their diagnosis using DSM-V by a child neurologist and child psychiatrist. Neurotypical children were randomly selected from primary schools in Ibadan. Inclusion criteria include newly diagnosed subjects (cases and positive controls) in the age range 3–8 years, free of medication and other known diseases such as anemia, diabetes, inflammatory diseases and infection. Approval for this study was obtained from the UI/UCH joint Ethical Committee as well as the Oyo State Ministry of Health Ethical Board. Informed written assent and consent were taken from all subjects and their parents, respectively, before specimen collection.

Sample collection, processing and storage

About 5 mL of venous blood was drawn from participants at the time of recruitment into special metal-free plain tubes. Subsequently, 10 mL of mid-stream urine was collected into sterile urine bottle and stored at – 20 °C. Blood samples were allowed to clot and then centrifuged at 3000 rpm for 10 min. The serum was separated and stored at – 20 °C until assayed.

Sample analysis

All biochemical analyses were performed at the postgraduate research laboratory, Department of Chemical Pathology, UCH.

Total protein estimation using biuret method

Quantitative assessment of total protein and albumin in serum and urine was done using the biuret and bromocresol green methods. Following established protocol [17], 500 mL of biuret reagent was prepared by dissolving 4.5 g of sodium potassium tartrate, 1.5 g of

copper sulfate tetrahydrate and 2.5 g of potassium iodide in 200 ml of 0.2M NaOH and then make up to 500 ml using distilled water. The biuret assay was conducted by adding 1 mL of samples, standards and controls to 3 mL of biuret reagent, incubated at 37 °C for 30 min and then read spectrophotometrically at 540 nm.

Serum globulin estimation

The Calculated Globulin (CG) method was used to estimate the levels of globulin in serum and urine samples. This involved first determining the concentration of total protein and albumin in the serum. The globulin level was then calculated by subtracting the albumin concentration from the total protein concentration [18].

Urinary protein estimation using sulphosalicylic acid method

Quantitative assessment of the total protein in urine was performed using turbidimetric method (sulphosalicylic acid method) described by Varley (1954). 3.5 mL SSA was added to 0.5 mL urine samples, standards and controls. The solution was mixed, and absorbance was read at 660 nm against blank. A standard curve was plotted, and sample concentrations were intrapolated.

Albumin estimation using bromocresol green method

To prepare 100 ml of stock bromocresol green (BCG) reagent, the method outlined by Olaniyan et al [19] was followed. The preparation involved mixing 0.4 mL of 10.4% NaOH, 0.05g of BCG dye (Sigma Chemical Co. St. Louis, MO. USA), 0.10 mL of Tween 80, and 3 mL of 85% lactic acid, then diluting the mixture to 100 mL with distilled water. The pH of the reagent was adjusted to 4.0 by the drop-wise addition of 0.1N NaOH. The BCG working solution was prepared by diluting the stock solution (1:5) with distilled water. For the assay, 0.1 mL of serum and urine samples, along with standards and controls were added to 3 mL of the BCG reagent and then read spectrophotometry at 620 nm.

Serum and urine protein differentiation using cellulose acetate electrophoresis

Serum and urine electrophoresis were performed using 0.075M barbitone buffers at pH 8.6. Urine samples were first concentrated by centrifugation at 4000 rpm for 15 min. A 50 µL volume of each sample was then loaded onto the equilibrated cellulose acetate paper using 2 mm thick combs. The loaded paper was transferred into the electrophoretic tank where it was placed on the support medium for wick flow, with the origin toward the cathode. The tank was closed, and the red and black terminals were connected to appropriate terminals of the power supply. The electrophoresis, involving the migration of the protein toward the anode, was conducted at 160 V for 20 min. The electrophoresed proteins were stained using 0.1% ponceau in 5% acetic acid and subsequently destained with 5% acetic acid. Clear images of the electrophoretic separation were taken for further analysis.

Densitometric analysis

Densitometric analysis of electrophoresed protein was performed in this study using ImageJ installed on a Windows 10 64-bit. ImageJ, developed by National Institutes of Health (NIH), is a freely available image processing and analysis application commonly employed for various purposes in different life science fields, including medical imaging, microscopy, densitometry, western blotting and PAGE [20, 21]. This procedure involved using a rectangular selection tool to select lanes of interest to generate lane profile plots. Straight line selection tool was then selected to draw base lines and/or drop lines to ensure each peak of interest defines a closed area. The size of each peak was then measured by clicking inside the closed area with the wand tool. Each measured peak was labeled with its size as a percent of the total size of the measured peaks. The data were transferred to spreadsheet for further analysis. To express each band in globulin region in concentration, the percentage density of each band was multiplied by the concentration of serum globulin divided by the density of the all globulin bands of the lane (for a sample). The formula is illustrated as follows:

$$\text{Band concentration (e.g., } \alpha 1\text{-globulin)} = \frac{\text{band } (\alpha 1\text{-globulin}) \text{ intensity } \times \text{ serum globulin concentration (g/dl)}}{\text{intensity of all bands } (\alpha 1, \alpha 2, \beta \text{ and } \gamma \text{ globulin}) \text{ in the globulin region}}$$

Table 1 Comparison of biodata variables (age, weight, height and BMI) between cases and controls using ANOVA

Variables	Cases	Positive controls	Negative controls	*F* test	*P* value
Age (years)	5.13 ± 2.23	7.25 ± 4.33	7.00 ± 3.33	0.924	0.411
Weight (kg)	19.62 ± 6.30	22.32 ± 13.34	22.73 ± 4.27	0.289	0.752
Height (cm)	109 ± 18.09	119.43 ± 26.86	124.00 ± 10.29	1.229	0.313
BMI (kg/m^2)	16.32 ± 0.76	14.72 ± 2.75	14.99 ± 1.30	2.078	0.150

The values of age, weight, height and BMI were expressed as mean ± standard deviation

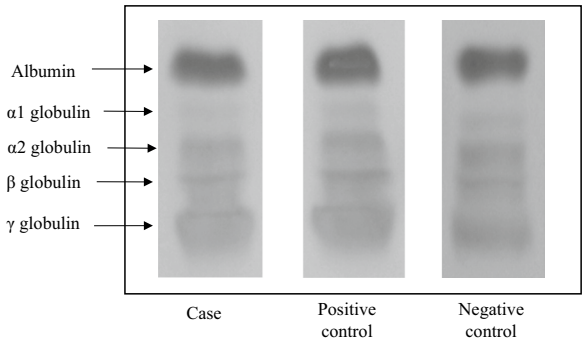

Fig. 1 A representative electrophoretic pattern of serum protein fractions for all groups

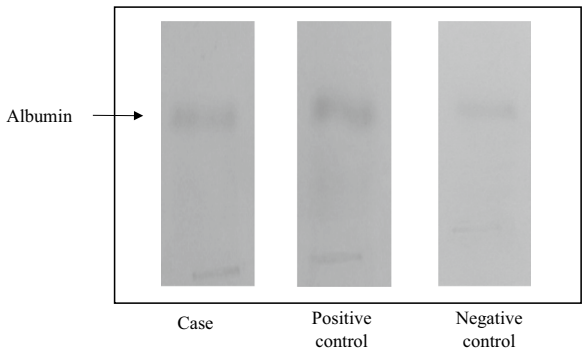

Fig. 2 A representative electrophoretic pattern of urine protein fraction for all groups

Statistical analysis

Using SPSS version 25, appropriate statistical analysis was undertaken. Specifically, the normal distribution of the data was assessed using Kolmogorov–Smirnov and Shapiro–Wilk tests. Mean ± SD was used to express data with normal distribution. ANOVA was used to compare the means among three groups and a post-hoc test (Tukey's HSD Test) was used to identify the significant pair(s). Kruskal Wallis test was used for data that violate the assumption of one-way ANOVA. Pearson correlation was used to determine the correlation between the studied variables. Differences between groups at $p < 0.05$ were regarded as significant.

Result

The anthropometric characteristics of study participants are summarized in Table 1, showing the mean age, weight, height and BMI of the cases and controls. Specifically, the mean ages were 5 ± 2.3 years, 7 ± 3.3 years and 7.4 ± 2.8 years for the case, positive controls and negative controls, respectively. Furthermore, the weight (kg), height (cm) and BMI (kg/m^2) were highest in negative controls (22.73 ± 4.27, 124.00 ± 10.29 and 16.32 ± 0.76, respectively), followed by the positive controls (22.32 ± 13.34, 119.43 ± 26.86 and 14.72 ± 2.75, respectively) and then the cases (19.62 ± 6.30, 109 ± 18.09 and 14.99 ± 1.30, respectively). However, no statistically significant differences were observed in the average age, height, weight and BMI across the three groups.

Representative electrophoretic patterns of serum and urine protein fractions for all studied groups are shown in Figs. 1 and 2, respectively. While both the case and control groups displayed a fairly typical and uniform serum electrophoretic pattern, clearly depicting five protein bands—albumin, alpha1 globulin, alpha2 globulin, beta globulin, and gamma globulins—there were slight variations in intensity and distribution among the groups. Similarly, the urine electrophoresis results revealed a pattern consistent with normal urine, characterized by a faint albumin band of varying intensities across the three groups.

Furthermore, Table 2 present the values of serum and urine total proteins and their fractions. According to the result, there was no significant difference in the diagnostic parameters of the three groups except for alpha2 globulin, gamma globulin and urine protein. Specifically, a Kruskal–Wallis H test (nonparametric one-way ANOVA) showed that there was a significant difference in urine total protein between the different groups, $\chi^2(2) = 6.301$, $p = 0.043$, with a mean rank urine protein level of 30.11 for cases, 20.88 for positive control and 14.00 for negative control.

Table 2 Comparison of serum and urine proteins (Mean ± SD) between cases and controls using *F* test

Variables	Cases	Positive controls	Negative controls	*F* test	*P* value
Serum total protein (g/dL)	7.79 ± 0.62	7.32 ± 0.93	7.63 ± 0.52	0.939	0.399
Serum albumin (g/dL)	4.62 ± 0.80	4.07 ± 0.75	4.55 ± 0.54	2.928	0.064
Serum globulin (g/dL)	3.17 ± 0.58	3.25 ± 0.66	3.08 ± 0.54	0.309	0.736
Alpha1 globulin (g/dL)	0.60 ± 0.05	0.59 ± 0.11	0.57 ± 0.09	0.194	0.826
Alpha2 globulin (g/dL)	0.52 ± 0.05	0.47 ± 0.11	0.65 ± 0.12	4.827*	0.024
Beta globulin (g/dL)	0.57 ± 0.09	0.47 ± 0.11	0.55 ± 0.09	1.712	0.214
Gamma globulin (g/dL)	2.05 ± 0.17	1.82 ± 0.37	1.23 ± 0.31	12.23*	0.001
Serum albumin/globulin (A/G) ratio	1.54 ± 0.55	1.30 ± 0.37	1.54 ± 0.43	1.455	0.244
Urine protein (mg/dL)	6.65 ± 3.06	4.32 ± 1.02	2.61 ± 1.90	12.77[a]	0.002*
Urine albumin (mg/dL)	1.28 ± 0.12	1.26 ± 0.13	1.29 ± 0.08	1.233	0.303

Independent sample Krukal Wallis *H* test score

*The mean difference is significant at $P < 0.05$

Table 3 Post hoc comparison of *α*2 and *γ* globulin between groups using Tukey HSD

Variables	Groups	Mean difference	*P* value
Alpha2 globulin (g/dL)	Cases vs positive controls	0.04667	0.709
	Cases vs negative controls	− 0.12833	0.104
	Positive controls vs negative controls	− 0.17500*	0.023
Gamma globulin (g/dL)	Cases vs positive controls	0.23000	0.389
	Cases vs negative controls	0.81500*	0.001
	Positive controls vs negative controls	0.58500*	0.010

*The mean difference is significant at $P < 0.05$

Table 4 Post hoc analysis of difference in urine protein between groups using Dunn test

Variables	Groups	Test statistic	SE	Std test statistic	*P* value
Urine Protein (mg/dL)	Cases versus positive controls	6.885	4.604	1.495	0.404
	Cases versus negative controls	16.107	4.515	3.568*	0.001
	Positive versus negative controls	9.223	4.679	1.971	0.146

*The mean difference is significant at $P < 0.05$

Post-hoc analysis of significant variables revealed a significant mean difference in alpha-2 globulin levels only between the positive and negative control groups, and in gamma globulin levels between the ASD group and the negative controls, as well as between the positive and negative controls (see Table 3).

Following the post-hoc Dunn test for urine protein levels, a significant difference in mean was observed only between the ASD group and the negative controls (see Table 4).

To explore the associations between the parameters investigated in this study, Pearson's correlation analysis was performed. The results of these relationships are presented in Table 5. Notably, significant correlations were observed between β globulin and urine microalbumin (UMA) in cases, between urine protein (UP) and serum globulin (SG) in positive controls, and between serum albumin globulin ratio (SAG) and gamma globulin (γ glb) for negative controls.

Discussion

This study aimed to contribute to the growing understanding of immune dysfunction in the pathophysiology of ASD by investigating protein separation patterns in Nigerian children with ASD using cellulose acetate electrophoresis. To achieve this objectives, 16 ASD children diagnosed by a child psychiatrist using DSM-5 were recruited from the Department of Child and Adult Psychiatry, UCH, Ibadan. The anthropometric characteristics of these cases were not significantly different from

Table 5 Correlations between studied variables among cases, positive controls and negative controls

Variables	Cases		Positive controls		Negative controls	
	r	*P* value	*r*	*P* value	*r*	*P* value
STP/UP	− 0.167	0.569	0.267	0.377	− 0.447	0.126
STP/UMA	− 0.341	0.234	0.200	0.513	− 0.137	0.656
STP/SAG	− 0.197	0.500	− 0.045	0.883	− 0.229	0.451
UP/SG	− 0.230	0.429	0.602*	0.029	− 0.503	0.079
UP/SA	0.080	0.785	− 0.198	0.517	0.086	0.779
UP/SAG	0.120	0.684	− 0.504	0.079	0.441	0.132
SG/UMA	− 0.283	0.327	0.537	0.059	− 0.570*	0.042
SA/UMA	− 0.037	0.899	− 0.232	0.445	0.436	0.136
UMA/SAG	0.129	0.660	− 0.505	0.079	0.652*	0.016
α1 glb/UP	− 0.654	0.159	− 0.545	0.264	− 0.695	0.125
α1 glb/UMA	− 0.007	0.99	0.137	0.795	0.018	0.972
α1 glb/SAG	− 0.642	0.169	− 0.892*	0.017	− 0.595	0.213
α2 glb/UP	− 0.283	0.587	0.419	0.408	− 0.621	0.188
α2 glb/UMA	0.062	0.908	− 0.385	0.450	0.335	0.517
α2 glb/SAG	− 0.363	0.479	− 0.305	0.556	− 0.980**	0.001
β glb/UP	0.330	0.523	0.438	0.384	− 0.288	0.580
β glb/UMA	0.822*	0.045	− 0.400	0.432	0.186	0.724
β glb/SAG	− 0.480	0.336	− 0.302	0.561	− 0.599	0.209
γ glb/UP	− 0.217	0.68	− 0.735	0.096	− 0.539	0.270
γ glb/UMA	0.436	0.388	0.475	0.341	0.335	0.517
γ glb/SAG	− 0.679	0.138	− 0.801	0.056	− 0.948**	0.004

'*r*' indicates Pearson coefficient, and asterisks ** and * represent significance levels at 1% and 5%, respectively

STP serum total protein, *UP* routine urine protein, *SG* serum globulin, *SA* serum albumin, *SAG* serum albumin/globulin ratio, *UMA* urine microalbumin, *α1 glb* alpha1 globulin, *α2 glb* alpha2 globulin, *β glb* beta globulin, *γ glb* gamma globulin

those of controls. This use of age-matched controls in this study enables reasonable comparison of biochemical parameters between cases and controls with close biological makeup, reducing the influence of confounding variables on our results [22].

The analysis of serum protein profiles revealed interesting findings. While the overall protein profiles between cases and controls were largely similar, significant differences emerged in specific fractions. Children with ASD exhibited significantly higher gamma globulin levels compared to neurotypical children. Interestingly, children with other NDDs in the control group showed significantly lower alpha1 globulin levels compared to the neurotypical group. Similarly, the trend toward lower albumin levels in the positive control group compared to the ASD and typically developing groups raises interesting questions about the role of serum albumin in NDDs. Further studies with larger sample sizes are needed to explore whether these trends could become significant and to understand the underlying mechanisms that may contribute to these differences. These findings highlight the potential for distinct protein profiles associated with different neurodevelopmental conditions. Studies on other psychological disorders support this notion. For example, lower serum total protein and albumin but increased serum alpha1 globulin was observed in patients with major depression [23]. Similarly, increased levels of serum total protein and various globulins (alpha1, alpha2, beta, and gamma) have been associated with psychological stress [24]. In the context of autism, Croonenberghs, Wauters [15] reported a unique protein profile in a Belgian population, characterized by elevated serum total protein, albumin, gamma globulin, and specific IgG subclasses (IgG2 and IgG4). Our study partially aligns with their findings regarding hypergammaglobulinemia in children with ASD compared to controls. However, it contrasts the findings of ElBaz, Zaki [16] who found no significant difference in serum protein levels between autistic children and healthy controls in Egypt.

The underlying mechanisms for the observed hypergammaglobinemia in our ASD group remain unclear. However, several potential explanations can be explored. One possibility involves autoimmune disorders, linked to NDDs, including ASD [4], which is often accompanied by a raised gammaglobulinemia resulting from polyclonal B-cell activation [25]. Apart

from antibodies, proteins with gamma mobility (e.g., complement products) could also contribute to the increased gamma globulin [15]. Exposure to viral infections congenitally or during postnatal period have been implicated in the incidence of ASD [4]. Such chronic infectious state or increased susceptibility to infections may also contribute to increased gamma globulin observed in autistic children compared to neurotypical children. Furthermore, autistic children often presents with comorbidities such as gastrointestinal problems characteristic of inflammatory bowel disease [26]. This condition is accompanied with an increase in specific IgG subclasses [27], thereby raising gamma globulin concentration. These potential explanations for hypergammaglobulinemia in ASD require further investigation.

Another unique finding of this study involved the observation of lower alpha2 globulin in children with ASD and related NDDs compared to neurotypical controls. The difference, however, was statistically significant only between the NDD group and neurotypical children. While specific reasons remain unclear, this findings might be related to gastrointestinal problems, particularly Inflammatory Bowel Disease. This condition, commonly associated with NDDs, causes damage to the intestinal lining, resulting in the loss of blood proteins, such as alpha2 globulins [28]. Low alpha2 globulin can also occur in cases of malnutrition, megaloblastic anemia, and Wilson's disease [29], conditions that may be particularly relevant to neurodiverse children due to their selective dietary habits. This observation contrasts with past research by Cortelazzo, De Felice [30], which found an elevated alpha2 globulins, particularly alpha2 macroglobulin, in ASD patients compared to controls. Alpha2 globulins play a crucial role in regulating immune response. For instance, alpha2 macroglobulin, due to its proteolytic activity, inhibit proteases released by neutrophils during inflammation or by invading microorganisms [31]. Therefore, the observed low alpha2 globulins in our study might indicate potential immune dysregulation in autistic children, warranting further exploration.

Beyond serum analysis, urine analysis offers a valuable non-invasive tool for assessing a wide range of plasma proteins, including low-molecular-weight proteins and various peptides [32]. This method can potentially reveal biomarkers for diseases not primarily affecting the kidneys, as pathological changes in the kidney's microvascular architecture often reflect systemic conditions. In our study, consistent with previous studies reporting higher protein excretion in autistic children [33, 34], we found significantly higher random urine protein levels in the ASD group compared to controls. This finding aligns with the frequent occurrence of gastrointestinal symptoms in autistic children, such as inflammation, diarrhea, and increased gut permeability, which allows smaller proteins and peptides to enter the bloodstream and be excreted in the urine [33].

Interestingly, despite the higher overall protein levels, urinary albumin excretion was similar across ASD and control groups in our study. This suggests that the elevated protein levels in ASD are likely due to the excretion of smaller, non-albumin proteins [34]. Furthermore, our use of cellulose acetate electrophoresis (CAE) for urinary protein separation revealed a normal urine pattern, with only faint albumin bands observed in all groups. This finding indicates that potential protein biomarkers in the urine of ASD children might be below the detection threshold of CAE, a technique known for its limitations in resolving heterogeneous proteins [35]. The higher spot urine protein levels in ASD children do not necessarily point to kidney pathology, as no significant differences were found in urine albumin levels between the ASD, positive control, and negative control groups. Albumin is typically the primary protein excreted in pathological kidney conditions; therefore, the absence of elevated albumin suggests that the increased protein excretion in ASD is likely not due to glomerular damage [36].

Several explanations could explain this observation. First, ASD has been linked to low-grade inflammation in the brain and body [9, 26]. This could lead to increased permeability of the glomerular filtration barrier in the kidneys [37], allowing non-albumin proteins, such as low molecular weight proteins, to pass into the urine [33]. Second, the unique behaviors often observed in ASD—such as hyperactivity, repetitive movements, and heightened stress responses—could contribute to transient increases in protein excretion, such as in exercise-induced proteinuria [38]. Third, differences in protein metabolism or turnover rates in children with ASD, possibly influenced by their selective dietary habits, could result in increased urinary excretion of specific proteins, contributing to the higher overall protein levels [39]. These metabolic variation might be independent of any renal pathology and could be linked to the unique physiological or nutritional profiles often seen in ASD. Nevertheless, the observed difference in overall urine protein levels underscores the need for further investigation into the urine proteome of ASD children using more advanced protein analysis techniques. This could potentially lead to the identification of novel urine protein biomarkers for ASD.

The correlation analysis revealed interesting associations between various protein fractions and

urinary measures across the groups. Notably, a significant positive correlation was observed between urine protein and serum globulin in children with NDDs (excluding ASD). Similarly, the autistic group exhibited a significant positive correlation between beta globulin and urine microalbumin. These finding suggests a potential link between overall protein metabolism and urinary protein excretion in these children. Further investigation is warranted to elucidate the specific mechanisms underlying these relationships.

Conclusion

This study contributes to the growing body of research linking immune dysfunction to ASD by investigating the concentration and electrophoretic patterns of serum and urine proteins in autistic children compared to neurotypical controls. Our key finding revealed significantly higher gamma globulin levels in autistic children, potentially implicating autoimmune disorders or chronic infections in ASD development. Additionally, the shared observation of elevated gamma globulin in children with other NDDs suggests a possible common immunological thread across these conditions. Furthermore, the observed reduction in alpha-2-globulins in children with NDDs compared to controls might indicate potential immune dysregulation in NDDs. Together, these findings suggest that abnormalities in immune-related proteins, characterized by increased gamma globulin and reduced alpha-2-globulin levels, could be indicators of NDDs, particularly ASD. We also observed a significant increase in urinary protein concentration in autistic children compared to controls. This finding aligns with previous research suggesting the potential of smaller proteins and peptides in urine as biomarkers for ASD.

Limitations and future directions

Despite its contributions, this study has limitations that offer opportunities for future research. First, the sample size, while exceeding the minimum requirement, was relatively small (16 participants per group). A larger sample size could strengthen the generalizability of our observations. Second, the study did not match case subjects with controls by sex, which could have introduced some variability. Future studies should consider sex-matched groups to improve statistical efficiency. Additionally, the study population was limited to a single location in Nigeria. Further research with more diverse populations is necessary before generalizing the findings. Future studies could benefit from employing more advanced proteomic techniques to investigate the nature and functions of proteins excreted by autistic children. This could potentially lead to the identification of specific protein biomarkers for use in ASD diagnosis and monitoring.

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Author contributions
IOO conceived the research concept and provided oversight throughout the analysis process. MAA conducted the analysis, interpreted the sample data, and drafted the manuscript. OAO contributed to both sample collection and analysis, as well as manuscript writing. YCA supervised the sample collection and provided critical revisions to the manuscript. All authors reviewed and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Approval for this study was obtained from the UI/UCH joint Ethical Committee as well as the Oyo State Ministry of Health Ethical Board. Informed written assent and consent were taken from all subjects and their parents, respectively, before specimen collection.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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