Value of neutrophil gelatinase-associated lipocalin in the diagnosis of renal disorders in children with glycogen storage disease

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Purpose
This study aimed to evaluate the value of urinary neutrophil gelatinase-associated lipocalin (NGAL) in the diagnosis of renal disorders in children with glycogen storage disease (GSD).
Patients and methods
This cross-sectional study, which was conducted at Benha University Hospital, Egypt, included 50 children with GSD and apparently healthy 50 children as a control group. The level of NGAL was measured in urine by enzyme-linked immunosorbent assay and compared between groups.
Results
The GSD group had statistically significant higher urinary NGAL (147.6±38.8 ng/ml) compared to the control group (102.1±11.4 ng/ml, $P<0.001$). Urinary NGAL was higher in patients with microalbuminuria, kidney injury (RIFLE >2), and patients with a higher degree of liver fibrosis. At a cutoff point more than or equal to 154 ng/ml, urinary NGAL could detect cases with microalbuminuria with a sensitivity of 83.3% and specificity of 94.7%. At a cutoff point more than or equal to 172 ng/ml, urinary NGAL could detect cases with acute kidney injury (RIFLE >2), with a sensitivity of 100% and specificity of 100%.
Conclusion
Urine NGAL demonstrates superior efficacy as a biomarker for the precocious identification of renal dysfunction in pediatric patients afflicted with GSDs.

Keywords:
children, glycogen storage diseases, neutrophil gelatinase-associated lipocalin, renal disorders

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Introduction

Glycogen storage diseases (GSD) represent a spectrum of inherited metabolic disorders primarily characterized by autosomal recessive transmission, which disrupts the pathways of glucose homeostasis and glycogen synthesis. These disorders manifest predominantly in the hepatic and muscular systems and have variable severity. Several GSDs exhibit renal manifestations; for instance, GSD type I is frequently associated with renal tubular dysfunctions, nephrolithiasis, and the development of chronic kidney disease (CKD) akin to the renal complications observed in diabetes mellitus [1].

The initial documentation of renal dysfunction emerging in cases of GSD-I was reported by Chen *et al.* [2]. Nephropathy associated with GSD is a commonly observed complication, likely attributable to a primary enzymatic deficiency within the renal architecture or secondary to the altered metabolic milieu ensuing from hepatic enzyme deficits. Nevertheless, controlling these metabolic aberrations can diminish the prevalence of renal impairment. Additionally, research has demonstrated that GSD-I patients receiving dietary intervention early in their disease course exhibit reduced proteinuria compared to those undergoing delayed treatment, implying that early correction of metabolic disturbances may either prevent or decelerate renal disease progression [3].

Upon the onset of proteinuria, therapeutic intervention with angiotensin-converting enzyme inhibitors proved inadequate in mitigating the inexorable advancement toward terminal renal failure. Renal lesion constitutes a principal complication, initially presenting as an elevated glomerular filtration rate (GFR), subsequently transitioning to microalbuminuria, and then to a diminished GFR accompanied by proteinuria. This pathological progression may further exacerbate renal interstitial fibrosis and glomerulosclerosis, ultimately

resulting in renal failure. Therefore, the early identification and prompt management of renal impairment are critical [4].

Neutrophil gelatinase-associated lipocalin (NGAL) belongs to the lipocalin family and is typically expressed at minimal levels across various human tissues. It is swiftly secreted from renal tubular cells as an acute response to assorted renal insults [5]. Serum and urinary levels of NGAL are increasingly recognized as the most promising biomarkers for the early identification of acute kidney injury (AKI) [6]. Recent research has established that NGAL serves as a significant prognostic marker for AKI in pediatric populations across diverse clinical contexts [7].

Recent research has highlighted that measurements of NGAL in both serum and urine are indicative biomarkers for the evaluation of disease presence and progression in CKD [8].

Aim

This study aims to assess the diagnostic utility of urinary NGAL in identifying renal disorders in pediatric patients with GSD.

Patients and methods

This cross-sectional, case–control study was executed at the Pediatric Hepatology Unit of Benha University Hospitals in Benha, Egypt, spanning from January 2022 to April 2024, and included 50 children with GSD (GSD group); 27 (54%) males and 23 (46%) females, their mean age was 9.1±4.2 years, and apparently healthy 50 children: 25 (50%) males and 25 (50%) females, their mean age was 10.3±3.9 years, matched for age and sex of studied group as a control group (N9 000476, date 25/3/2023). Any child with GSD with associated comorbidities like cardiac, renal, etc., were excluded from the study.

Each participant was subjected to an extensive protocol that encompassed the collection of their complete medical history, a thorough physical examination, and a series of laboratory tests to evaluate their blood count, kidney and liver function, and urinary NGAL levels.

Blood sample collections

Complete blood count for all specimens was conducted utilizing the automated hematology analyzer, XS series, model SN 12526, manufactured by SYSMEX Corporation (Kobe, Japan).

Coagulation function (prothrombin time, partial thromboplastin time, and international normalized ratio) using automated blood coagulation analyzer CS-1600, SN 12058, Sysemex Corporation.

Biochemical liver and kidney function tests were assessed by DIALAB, 13771103, Thermo Company (Made in China, High quality, First Class).

Gamma glutamyl transferase was assessed by INDIko, SN 864000003200, Thermo Company.

Sodium, potassium, and calcium were assessed by ST 200 Plus, Sensacore, SN IVD5214732, Thermo Company.

Urine sample collections

Urine samples were withdrawn and collected in a clean container:

(1) Protein and albumin levels in urine were quantified utilizing the TBA-200FR analyzer produced by Toshiba Medical Systems, based in Tokyo, Japan.
(2) Urinary NGAL quantification was performed concurrently using the human (NGAL) enzyme-linked immunosorbent assay Kit, catalog No. 201-12-1720, and readings were taken with a DAS plate reader, serial number 1912, located in Palombara Sabina, Italy.

The GFR level was calculated using the MDRD formula: GFR=186×[serum creatinine (mg/dl)] $-1.154\times age^{-0.203}$ [9].

Abdominal ultrasonography was done for all patients to assess liver size and kidney size and echo pattern, texture, spleen size, and presence of ascites. A Liver biopsy was performed on all patients. This process was undertaken to assess the histological activity of hepatitis employing the modified Ishak *et al.* [10] scoring system. Ascertainment of AKI through the application of the RIFLE criteria framework [11].

Ethical considerations

The entire research protocol received approval from the local ethics committee (MS 11-3-2021) at the Faculty of Medicine, Benha University. Throughout all phases of the study, strict confidentiality and personal privacy were upheld. Guardians retained the right to withdraw their participation at any stage without any repercussions. Additionally, the data collected were exclusively used for this study and are not intended for any alternate purposes.

Statistical analysis

The data acquisition and analytical processes were executed employing SPSS software, version 16 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were characterized by their means±SDs and range values. In contrast, categorical variables were delineated via their respective frequencies and percentages. The analysis of categorical data employed the χ^2 test and Fisher's exact test, whereas the Shapiro–Wilks test was utilized to evaluate the normality of quantitative data, with normality presumed at P value more than 0.05. The Student's t test was applied to analyze normally distributed continuous variables between two independent groups. Spearman's correlation coefficient (rho) was employed to determine correlations among nonparametric variables. Receiver operating characteristic (ROC) curve analysis was conducted to ascertain cutoff values for the markers under study, aiming to achieve optimal sensitivity and specificity. A significance threshold was established at P value less than 0.05, with values below this threshold deemed statistically significant.

Results

The GSD cohort exhibited a statistically higher incidence of positive family history for GSD than the control group. Conversely, the two groups were matched in regard to age, sex, or consanguinity (Table 1).

The most common complaint in the studied group was abdominal distension (34%), followed by hypoglycemia (34%), failure to thrive (20%), and abdominal pain (12%). Regarding other symptoms, 26% had pallor, 16% had jaundice, 4% had melena, 4% had hematemesis, and none of the cases had easy bruising, oliguria, pruritus, nausea, and vomiting, or ascites. No abnormalities in urine and stool color were detected. Liver biopsy showed that most cases (62%) had mild fibrosis, while 20% had moderate fibrosis and 8% had severe fibrosis. Regarding the Hepatic Activity Index (HAI) score, 14% of patients were minimal, 62% of patients were mild, 20% were moderate, and 4% were severe.

GSD had a statistically higher frequency of abnormalities in urine analysis, microalbuminuria, and higher RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) score compared to the control group. While there was no significant difference between groups as regards kidney ultrasonography (Table 2).

The GSD group had statistically significant higher urinary NGAL (147.6±38.8 ng/ml) compared to the control group (102.1±11.4 ng/ml, $P<0.001$) (Fig. 1).

Patients exhibiting microalbuminuria demonstrated statistically significant elevated levels of urinary NGAL in comparison to those without microalbuminuria. Patients with kidney injury had statistically significantly higher levels of urinary NGAL compared to patients with risk and no risk at RIFLE score. Patients with liver fibrosis and higher degree of HAI had statistically significant higher levels

Table 1 Sociodemographic data of the studied groups

	Groups [n (%)]			
	GSD group (N=50)	Control group (N=50)	Test	P value
Age (years)				
Mean±SD	9.1±4.2	10.3±3.9	t=1.4	0.114
Range	3–17	3–17		
Sex				
Male	27 (54.0)	25 (50.0)	χ^2=0.97	0.31
Female	23 (46.0)	25 (50.0)		
Consanguinity				
Negative	26 (52.0)	29 (58.0)	χ^2=0.36	0.54
Positive	24 (48.0)	21 (42.0)		
Family history of liver diseases				
Negative	37 (74.0)	50 (100.0)	χ^2=14.9	<0.001*
Positive	13 (26.0)	0		
Age of disease onset (years)				
Mean±SD	1.9±1.2			
Range	6 months–5 years			
Duration of disease (years)				
Mean±SD	7.2±4.1			
Range	2–15			

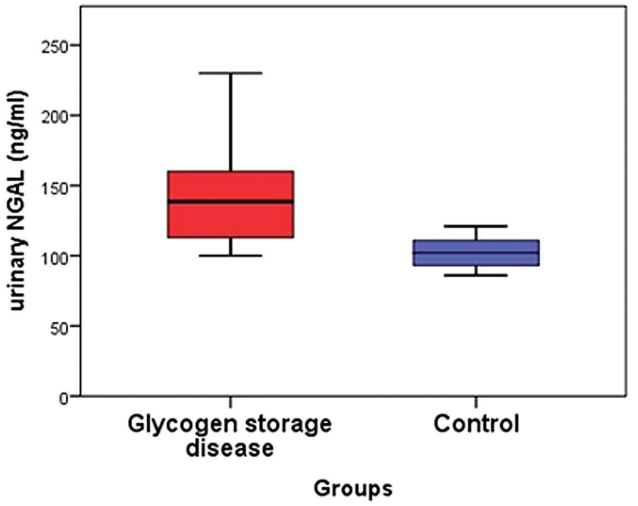
χ^2, χ^2 test; GSD, glycogen storage disease; t, Student t test. *Significant.

Table 2 Kidney assessment in the studied groups

	Groups [*n* (%)]			
	GSD group (*N*=50)	Control group (*N*=50)	Test	*P* value
Kidney ultrasonography				
Normal	50 (100.0)	50 (100.0)	–	–
Abnormal	0	0		
Urine analysis				
Albumin	12 (24.0)	0	χ^2=10.8	<0.001*
Calcium	4 (8.0)	0		
Glucose	6 (12.0)	0		
No abnormalities	36 (72.0)	50 (100.0)		
Microalbuminuria				
No	38 (76.0)	50 (100.0)	χ^2=11.9	<0.001*
Yes	12 (24.0)	0		
RIFLE score				
No risk	29 (58.0)	50 (100.0)	χ^2=32.6	<0.001*
Risk	7 (14.0)	0		
Injury	14 (28.0)	0		

χ^2, χ^2 test; GSD, glycogen storage disease; RIFLE; Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease. *Significant.

Figure 1



Urinary NGAL in the studied groups. NGAL, neutrophil gelatinase-associated lipocalin.

Table 3 Urinary neutrophil gelatinase-associated lipocalin level as regards liver and kidney affection

	Urinary NGAL (ng/ml)			
	Mean±SD	Range	Test	*P* value
Microalbuminuria				
No	114±23	86–214	*t*=12.4	<0.001*
Yes	202±24	160–230		
RIFLE score				
No risk	111±17	86–144	*F*=19.8	<0.001
Risk	123±15	103–137		
Injury	203±23	160–230		
Liver fibrosis				
Mild	141±35	100–222	*t*=4.1	0.001*
Moderate	214±18	198–230		
HAI				
Minimal	143±39	100–230	*F*=8.6	0.008*
Mild	160±35	137–211		
Moderate	194±10	178–198		

F, *F* value of one-way analysis of variance; HAI, Hepatic Activity Index; NGAL, neutrophil gelatinase-associated lipocalin; RIFLE, Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease; *t*, Student *t* test. *Significant..

of urinary NGAL compared to patients with mild fibrosis and lower degree of HAI (Table 3).

Significant positive correlations were identified between urinary NGAL and the biomarkers alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, international normalized ratio, urea, and creatinine. In contrast, significant negative correlations were observed between urinary NGAL and random blood sugar, albumin, and GFR. No significant correlations were detected between urinary NGAL and hemoglobin, white blood cells, platelets, gamma glutamyl transferase, both forms of bilirubin (total and direct), total protein, prothrombin time, partial thromboplastin time, and both forms of calcium (total and ionized) (Table 4).

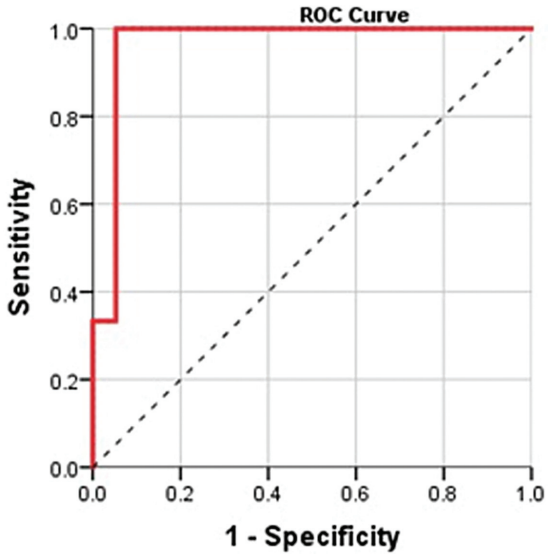
ROC analysis was conducted to evaluate the diagnostic capability of urinary NGAL in identifying microalbuminuria cases; the area under the curve (AUC) achieved was 0.965 (95% confidence interval: 0.915–1), with a significance level of *P* value less than 0.001. Utilizing a cutoff more than 154 ng/ml, the sensitivity registered at 83.3% and specificity at 94.7%, as depicted in Fig. 2.

Table 4 Correlation between urinary neutrophil gelatinase-associated lipocalin and laboratory investigations

	Urinary NGAL (ng/ml)	
	r	*P* value
Hemoglobin (mg/dl)	0.173	0.085
WBCs ($\times 10^3$/l)	0.050	0.618
Platelets ($\times 10^3$/l)	–0.076	0.453
AST (U/l)	0.331	0.001*
ALT (U/l)	0.353	<0.001*
ALP (U/l)	0.441	<0.001*
GGT (U/l)	0.113	0.133
PT (s)	0.103	0.309
PTT (s)	0.177	0.078
INR	0.460	<0.001*
RBS (mg/dl)	–0.223	0.026*
Total bilirubin (mg/dl)	0.166	0.081
Direct bilirubin (mg/dl)	0.138	0.176
Total protein (g/dl)	–0.126	0.211
Serum albumin (g/dl)	–0.380	<0.001*
Total calcium (mg/dl)	–0.101	0.316
Ionized calcium (mg/dl)	–0.017	0.868
Urea (mg/dl)	0.579	<0.001*
Creatinine (mg/dl)	0.835	<0.001*
GFR (ml/min/m^2)	–0.725	<0.001*

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GFR, glomerular filtration rate; GGT, gamma glutamyl transferase; INR, international normalized ratio; NGAL, neutrophil gelatinase-associated lipocalin; PT, prothrombin time; PTT, partial thromboplastin time; *r*, correlation coefficient; RBS, random blood sugar; WBCs, white blood cells. *Significant.

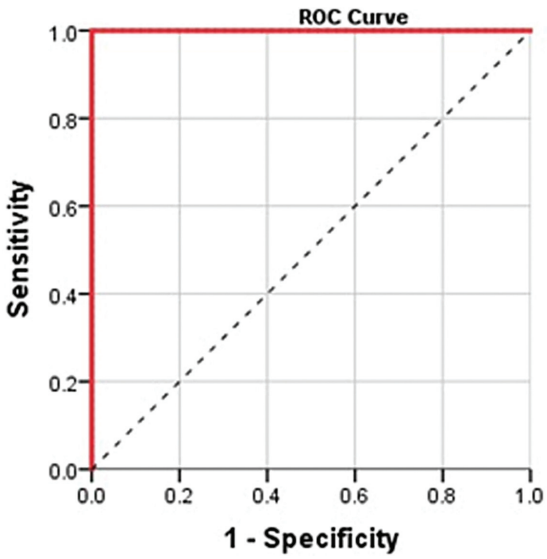
Figure 2



ROC curve of performance of urinary NGAL to detect cases with microalbuminuria. NGAL, neutrophil gelatinase-associated lipocalin; ROC, receiver operating characteristic.

ROC analysis was conducted to ascertain the efficacy of urinary NGAL in detecting cases of AKI with a RIFLE score exceeding 2. The AUC was perfect at 1 ($P<0.001$). Employing a threshold more than 172 ng/ml yielded both sensitivity and specificity at 100%, as illustrated in Fig. 3.

Figure 3



ROC curve of performance of urinary NGAL to detect cases with acute kidney injury. NGAL, neutrophil gelatinase-associated lipocalin; ROC, receiver operating characteristic.

Discussion

To the best of our knowledge, this study represents the inaugural study assessing urinary NGAL in pediatric patients with GSD. Nonetheless, previous studies examined the utility of NGAL in detecting renal impairment in individuals with CLD.

A preceding study in the adult population [12] scrutinized the quantification of serum and urinary NGAL in patients with chronic liver disease, evaluating its diagnostic value in identifying renal dysfunction. This study involved a cohort of 45 individuals suffering from chronic liver disease and revealed that both urinary and serum NGAL concentrations were substantially elevated in those with concomitant renal impairment, in contrast to their counterparts devoid of renal dysfunction ($P<0.05$). A multivariate analysis showed that the best predictor for hepatic patients with renal impairment was serum NGAL, followed by urinary NGAL. There was a positive correlation between serum and urine NGAL with serum creatinine, which correlated inversely with GFR. The best cutoff value of urinary NGAL to detect renal impairment in CLD was more than 343 ng/ml, AUC=0.78, with a specificity of 53.33% and a sensitivity of 93.33%.

Moreover, an alternate research group [13] embarked on an exploration of the applicability of urinary NGAL as a diagnostic biomarker for hepatorenal syndrome in the adult cirrhotic patient population. Their study revealed that urinary and plasma NGAL concentrations were markedly elevated in cirrhotic individuals with compromised renal function, irrespective of the presence of infection, in comparison to those exhibiting normal renal function.

In a distinct study [14], the utility of NGAL in forecasting mortality among adult patients with hepatorenal syndrome was examined. The findings revealed that individuals diagnosed with both type 2 and type 1 hepatorenal syndrome exhibited markedly elevated plasma and urine NGAL concentrations in comparison to patients with stable cirrhosis and controls.

Additionally, corroborating the findings of Alhaddad *et al.* [15], who researched NGAL as a novel indicator of renal function in hepatitis C-related end-stage liver disease, it was demonstrated that NGAL levels predict a GFR below 60 ml/min. Furthermore, serum creatinine levels exhibited a positive correlation with Model for End-Stage Liver Disease (MELD) and MELD-Na scores and a negative correlation with GFR, affirming NGAL's continued relevance as a renal function marker.

The results of our study are congruent with the findings of Zhang *et al.* [16], who examined the prognostic implications of NGAL in patients with type 2 hepatorenal syndrome. They ascertained that both plasma and urinary NGAL demonstrated a positive correlation with blood creatinine levels, the MELD score, the Child–Pugh score, and the presence of ascites.

Furthermore, another study [5], which assessed urinary NGAL concentrations relative to GFR for evaluating renal function in patients with diabetic CKD, concluded that there was a significant inverse relationship between GFR and urinary NGAL levels.

However, a previous study [15] that studied NGAL for assessment of renal function in C-related end-stage liver disease, found that the plasma NGAL level positively correlated with the GFR value measured by Renogram ($P<0.01$), unlike the urinary NGAL ($P=0.848$).

In a comprehensive study evaluating urinary NGAL as an early diagnostic marker for AKI in critically ill pediatric cohorts [17], the sensitivity parameters for urine NGAL fluctuated between 54 and 85%, while specificity parameters ranged from 97 to 44%. The study delineated an AUC of 0.79 for the prediction of AKI. At a urinary NGAL concentration threshold of 0.05 ng/mg creatinine, the specificity and sensitivity for predicting AKI within 48 h were 44 and 85%, respectively. At an elevated threshold of 1.5 ng/mg creatinine, specificity reached 97%, while sensitivity was 54%.

Urine and serum NGAL are increasingly being recognized as the foremost biomarkers for the early detection of AKI. Concurrently, numerous contemporary investigations have delineated NGAL's role within CKD, demonstrating that both serum and urinary NGAL concentrations serve as indicators of renal pathology and its severity in CKD [5].

Evidence indicates that NGAL is secreted from compromised renal tubular cells in the context of AKI preceding detectable reductions in GFR. Furthermore, urinary NGAL has been corroborated as a dependable early biomarker for diabetic nephropathy [18]. Urinary NGAL should be considered a relevant biomarker for the early detection of diabetic nephropathy during the normoalbuminuric phase, as suggested by the results of a meta-analysis that includes pediatric investigations. The pathophysiological development of diabetic nephropathy is recognized as being associated with injury to both the glomerular and tubular interstitial structures. In addition, research has shown that tubular dysfunction may occur prior to glomerular injury, resulting in the development of microalbuminuria [19].

Identification of urinary NGAL is attributed to absorption anomalies consequent to injury in both proximal and distal tubular segments. The paramount function of a biomarker such as NGAL lies in its capacity to discern individuals exhibiting subclinical AKI prior to an elevation in serum creatinine levels [20].

Conclusion

Urinary NGAL serves as an exceptional biomarker for the early detection of renal impairment in pediatric patients with GSD. However, further larger multicentric are needed to clarify the diagnostic and prognostic values of urinary NGAL in these patients.

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Competing interests

The authors declare no competing interests.

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