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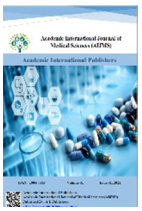

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Assessment of Neutrophil Gelatinase Associated Lipocalin in Children with Acute Gastroenteritis and Dehydration to Identify Early Signs of Acute Kidney Injury

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Abstract

Background: Gastroenteritis often correlates with acute kidney injury (AKI) in children who are hospitalized. The primary diagnostic test for acute kidney injury (AKI) in modern times is serum creatinine (SCr), which increases in the presence of AKI and is eliminated by glomerular filtration. SCr is an unsuitable biomarker for renal sickness because it lacks specificity and a slow response to disease severity or treatment changes. NGAL, or neutrophil gelatinase-associated lipocalin, is a molecular weight of 25 kDa protein and forms a covalent bond with neutrophil gelatinase. Elevations in NGAL levels due to kidney injury have important predictive value and may forecast the onset of acute kidney injury (AKI) 24-72 hours before an increase in diagnostic serum creatinine (SCr) values.

Aim and objectives: This study aims to determine whether plasma NGAL concentrations in mild, moderate, or severe dehydrated acute gastroenteritis patients may indicate acute kidney damage (AKI). The research will investigate whether acute renal injury and plasma NGAL concentrations are connected. Patients and methods: The cross-sectional design was employed in this study and included 80 patients who attended the pediatric gastrointestinal clinic at Babylon Children's Hospital. Between November 2022 and June 2023, all patients had gastroenteritis symptoms accompanied by different dehydration levels. Results: Patients with severe dehydration had considerable higher level of NGAL than those with mild to moderate dehydration ($p<0.001$). There was a notable inverse relationship ($p = 0.046$) between the NGAL level and potassium but a considerable direct link ($p<0.001$) between the NGAL level and creatinine. However, no significant correlation was seen between the NGAL level and urea ($p = 0.404$ and 0.062, respectively). The confidence range for the area under the curve (AUC) is 0.940 to 0.981, with a confidence level of 95%. The p-value is less than 0.001. The sensitivity is 88%. An accuracy of 88.4% has been attained. The NGAL cut-off point is 3.9832.

Conclusion: An analysis of plasma neutrophil gelatinase-associated lipocalin (NGAL) in individuals with gastroenteritis and varied degrees of dehydration indicated a clear and direct link between the two parameters. Specifically, when dehydration worsened, the average NGAL value increased.

Introduction

Acute diarrhea is a prevalent ailment in underdeveloped nations, often leading to hospital referrals. AKI is becoming more common in both developed and developing nations and hospitalized children often acquire gastroenteritis, which is the main factor contributing to AKI. [1]. There has been a rise in cases of acute renal injury, especially among the severely ill.

The practical outcome of a severe kidney injury is a reduction in glomerular filtration rate. Serum creatinine (SCr) is the most common way to diagnose acute kidney damage (AKI) today. It raises the risk of AKI and is removed from the body through glomerular filtration. [2]. "Human neutrophil gelatinase-associated lipocalin," or lipocalin 2, is a protein having a molecular weight of 25 kDa. It was initially discovered, and its covalent linkage with neutrophil gelatinase was later elucidated. The NGAL gene exhibits variable expression across numerous human organs, including the uterus, salivary glands, lungs, colon, and kidneys. The principal sources of its appearance are neutrophils and other epithelial cells.

Higher NGAL levels after kidney damage may indicate AKI 24 to 72 hours before the rise in diagnostic serum creatinine (SCr) levels, and they can also be used to predict how the patient will do [4]. This study aimed to determine if plasma NGAL levels can serve as an early marker for acute kidney injury (AKI) in patients with acute gastroenteritis and differing degrees of dehydration (moderate, mild, or severe).

Patients and Methods

Study population:

The study, which had a cross-sectional design, was carried out from November 2022 to June 2023. The sample comprised 80 patients visiting the paediatric "gastrointestinal clinic at Babylon Children's Hospital." Each patient had varying degrees of dehydration and gastrointestinal complaints. Patients were divided into three groups according to the severity of their dehydration: group C: severe, group B: moderate, and group A: mild.

Inclusion criteria

Age range: 2 months to 12 years, applicable to both genders, due to acute gastroenteritis. The WHO dehydration scale guidelines were used to establish the definition of dehydration: a reduction in total body weight of 3%–5% is classified as dehydration, a reduction of 6%–10% is classified as moderate dehydration, and a reduction of 10% or more is classified as severe dehydration. (WHO,2018).

Exclusion criteria

Known cases of chronic kidney disease, Chronic hepatic, intestinal, neurological, metabolic, or immunological diseases. Ethical considerations: The research followed the Helsinki Declaration. Parents of research children gave informed consent. Database processing secrecy was guaranteed. Participants' privacy was protected.

Methods

Each patient had a thorough assessment, including a detailed medical history, a complete physical examination, an evaluation of dehydration level, clinical symptoms, and a skin pinch test. The laboratory workup regimen includes routine laboratory investigations such as complete blood count, sodium, potassium, urea, creatinine, and C-reactive protein. The Schwartz formula provides eGFR, an estimate of the glomerular filtration rate. The value of eGFR (ml/min/1.73m2) is calculated as the ratio of 0.55 times the length (cm) to Scr (mg/dl) [5].

Measurement of NGAL biomarker:
The human serum was evaluated using the EIAab ELISA kit Cat# E1388h, which is an enzyme-linked immunosorbent assay (ELISA) kit. Statistical analysis was conducted by "MedCalc© version 18.2.1 (MedCalc©" Software4 Aca. Intl. J. Med. Sci. 2024; 1(2) 01-10 bvba, Ostend, Belgium) and IBM© SPSS© Statistics version 23 ("IBM© Corp., Armonk, NY"). Data adhering to a normal distribution were analyzed using a t-test. In a different method, the data were presented as the mean value, with the standard deviation indicated as a range. The "Mann-Whitney U test" was employed to analyze data that do not conform to a normal distribution. This test also determined the median and range. Pearson's correlation analysis was employed to calculate parameter correlations.

Results

Table (1): Demographic data of the study population

Age in months	**Group A**	**Group B**	**Group C**	**P- value**
Median (IQR)	32.5 (17.5:48)	9 (5:21)	9 (8:17)	0.287
Length in cm				
Mean± SD	80.8±17.7	71.7±19.9	66±12.2	
Median (IQR)	81 (68.5:93)	67 (58:79)	67 (59:73)	0.239
Weight in Kg				
Mean± SD				0.193
Gender 4/80	**39/80**	**37/80**		
male	3 (2.4)	26 (20.8)	22 (17.6)	0.72
Female	1 (0.8)	13 (10.4)	15 (12)	

There was no significant difference between the three groups as regards Age, Length, Weight, and Gender p= 0.287, 0.239, 0.193, 0.720 respectively. (Table 1)

Table (2): Symptoms and Laboratory results in patients with various degrees of dehydration

Degree of dehydration			
	Mild to Moderate dehydration	**Severe dehydration**	**P- Value**
Number of motions	**per day**		
Mean±SD	7.8±2.6	9.3±1.6	**0.035**
Median (IQR)	10(5:10)	10(10:10)	
Duration of illness in days			
Mean±SD			0.146
Median (IQR)	4(3:4)	4(3:7)	
Hemoglobin in gm/dl			
Mean±SD	9.9±1.2	10.2±1	0.566
Median (IQR)	10(9.4:10.7)	10.5(9.3:10.8)	
Total Leukocytic count in cells per liter			
Mean±SD			**0.006**
Median (IQR)	10.2(8:12)	12.4(10.3:14)	
Platelets per microlitre			

Mean±SD	267.8±96.1	281.9±92.5	0.648
Median (IQR)	260(226:318)	270(236:394)	
C Reactive Protein in mg/L			
Mean±SD			0.097
Median (IQR)	6(6:6)	6(6:1)	
Sodium in mEq/L			
Mean±SD	140±8.8	139.4±14.2	0.525
Median (IQR)	138(134:146)	137(128:145)	
Potassium in mEq/L			
Mean±SD	4.2±0.9	3.9±0.7	
Median (IQR)	4(3.6:4.7)	3.8(3.2:4.5)	0.286
Urea in mg/dl			
Mean±SD			
Median (IQR)	16(12:22)	25(15:34)	0.088
Creatinine in mg/dl			
Mean±SD			
Median (IQR)	0.5(0.4:0.6)	0.9(0.7:1.3)	**<0.001**
GFR in ml/min/1.73m²			
Mean±SD			
Median (IQR)	67.5(45:88.8)	30(15.4:37.8)	**<0.001**

Substantial variations were seen between the two groups in total leukocyte count, creatinine levels, glomerular filtration rate (GFR), and regular frequency (p=0.035, 0.006, p<0.001, p<0.001, respectively). The levels of hemoglobin, platelets per microlitre, C-reactive protein, sodium, potassium, and urea, as well as the duration of the illness in days, did not show significant variation (p=0.146, 0.566, 0.648, 0.097, 0.525, 0.286, 0.088, respectively) (Table 2).

Table (3): Comparison between the NGAL level in patients with various degrees of dehydration

	Mild to Moderate dehydration	**Severe dehydration**	**P- value**
NGAL in ng/ml			
Mean±SD	4.1±2	7±2.4	**<0.001**
Median (IQR)	4.2(2.5:5.7)	7.1(5.6:8.3)	

Data is shown as either mean ±SD or median (IQR)P- value <0.005 is significant

There was a substantial and reliable difference in the levels of NGAL among patients who were suffering varying degrees of dehydration, with a p-value of less than 0.001. (Table 3)

Table (4): Correlation between NGAL level and sodium, potassium, urea, creatinine

NGAL in ng/ml		
	r	**P value**
Na	-0.095	0.404
K	-.224*	**0.046**
Urea	0.209	0.062
Creatinine	.547**	**<0.001**

A substantial positive association existed between NGAL levels and creatinine (p<0.001), but a negative correlation with potassium (p=0.046). However, NGAL levels did not correlate with salt or urea levels (p-values of 0.404 and 0.062, respectively) (Table 4).

Table (5): Percentage of distribution and symptoms of Groups A, B, and C ofpatients

	Group A	**Group B**	**Group C**	
	N (%)	N (%)	N (%)	P- value
Gender	4/80	39/80	37/80	
male	3 (2.4)	26 (20.8)	22 (17.6)	0.720
Female	1 (0.8)	13 (10.4)	15 (12)	
Diarrhea				
watery	2 (1.6)	34 (27.2)	36 (28.8)	**0.008**
Well-formed	2 (1.6)	5 (4)	1 (0.8)	
Fever				
Yes	0 (0)	24 (19.2)	20 (16)	0.062
No	4 (3.2)	15 (12)	17 (13.6)	
Vomiting				
Yes	1 (0.8)	24 (19.2)	31 (24.8)	**0.014**
No	3 (2.4)	15 (12)	6 (12.8)	
Degree of Dehydration				
Mild	4 (3.2)	31(24.8)	0(0)	**<0.001**
Moderate	0 (0)	8(6.4)	22(17.6)	
Severe	0 (0)	0(0)	15(12)	

Data are represented as percentages. P- value <0.005 is significant.

There was significant difference between three groups as regards diarrhea, Vomiting and degree of dehydration p=0.008, 0.014, p<0.001 respectively, but there was no significant difference as regards Gender and Fever p=0.720, 0.062 respectively (Table 5)

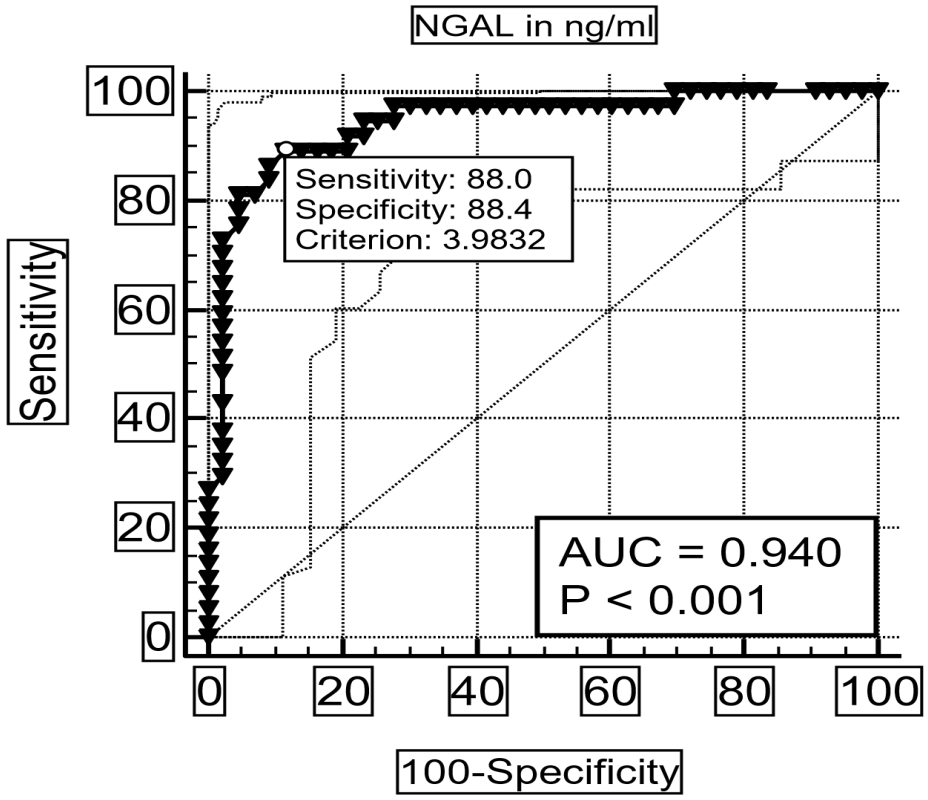

Figure (1): The NGAL biomarker's diagnostic potential is identified using a receiver-operating characteristic (ROC) curve.

Area under the ROC curve (AUC) = 0.940. 95% CI = 0.864 to 0.981. P-value <0.001. Sensitivity = 88%. Specificity = 88.4%. Cut-off point of NGAL =3.9832.

Discussion

Gastroenteritis-induced dehydration is a significant contributor to hospital admissions and illness severity in the pediatric emergency department (ED). Diarrheal disease is responsible for around 10% of deaths among children under the age of 5 worldwide [6].

The main results of our study were as follows:

Group 2 exhibited significantly higher creatinine levels (1.3 mg/dl (±1.2 mg/dl)) compared to group 1 (0.5 mg/dl (±0.2 mg/dl)) (p-value < 0.001).

Group 2's average GFR (31.4 ml/min/1.73m² (±17.3 ml/min/1.73m²)) was significantly lower than group 1's (73.8 ml/min/1.73m² (±37 ml/min/1.73m²)) (p-value <0.001).

Group 2 had significantly higher mean plasma NGAL biomarker levels (7 mg/dl; ±2.4 mg/dl) compared to group 1 (4.1 mg/dl; ±2 mg/dl) (p-value <0.001).

Dehydration, similar to other conditions that restrict blood flow to the kidneys, may decrease the pace at which blood is filtered in the glomerulus and harm the cells that line the glomerulus. The pre-renal stage is characterized by damage to the brush border of proximal tubule cells. Currently, routine blood creatinine testing is unable to identify minor cases of intense tubular necrosis. Dehydration, enzymes in the tubules, and high NGAL levels in the blood all point to pre-renal acute kidney injury [7]. NGAL levels rise quickly and significantly (within 2 hours) after injury, which could make it a valuable biomarker for quickly detecting moderate acute renal impairment, like what is seen in people with dehydrated gastroenteritis. The degree of dehydration had an impact on the severity of acute renal failure, and people with severe dehydration had higher NGAL concentrations than people who were just mildly or moderately dehydrated [8].

This result is consistent with research by Çelik et al., which looked at 35 healthy controls and 30 patients with mild to severe dehydration and diarrhea. The two groups' NGAL levels in plasma and urine were compared [7].

It was shown that the moderately dehydrated group had significantly higher levels of urine and plasma NGAL than people who were just mildly dehydrated ($p = 0.001$).

Antonopoulos et al. investigated twelve moderately dehydrated adults and twelve healthy controls. Their findings match ours. Dehydrated and control groups showed similar age, gender, and primary laboratory test results. Both groups included 75% men and comparable hemoglobin, white, red, urea, and creatinine levels. Although the two groups had similar laboratory results, dehydrated patients had considerably greater serum NGAL than controls ($p = 0.02$) [9].

Laboratory findings divided study subjects into three groups. Group A included 4 patients—5% of the total. Group B included 39 patients, 48.8% of the total. Group C included 37 patients, 46.3% of the total. Patients in Group A had normal GFR and undetectable NGAL levels of 0.156 to 3.9 ng/ml.

The average urea level in Group C was significantly higher than in Groups A (12.4 mg/dl (±4.6 mg/dl)) and B (19.2 mg/dl (±11.7 mg/dl)) (p-value 0.014). Group C exhibited significantly higher creatinine levels (1.01 mg/dl (±0.82 mg/dl)) compared to groups A (0.35 mg/dl (±0.06 mg/dl)) and B (0.41 mg/dl (±0.11 mg/dl)) (p-value <0.001).

The mean NGAL concentration in group C (6.53 ng/ml (±1.7 ng/ml)) was significantly higher than in groups A (2.11 /ml (±0.9 ng/ml)) and B (4.85 ng/ml (±0.8 ng/ml)) (p-value <0.00

A “receiver-operating characteristic” (ROC) curve set NGAL at 3.9832 ng/ml. Acute renal damage lowers glomerular filtration rate (GFR), which increases NGAL concentration.

The kidney, trachea, lungs, stomach, and colon release low-level human NGAL naturally. Damaged kidney, colon, and lung epithelial tissues produce NGAL. Recent studies and clinical evidence show that NGAL builds up in two storage sites during the early stages of AKI, which may result from a variety of causes: a kidney reservoir and a body reservoir. Research has demonstrated that acute kidney damage (AKI) increases NGAL mRNA expression in other organs, including the liver and spleen. Excess NGAL protein may enter the circulation and establish systemic storage [10]. Immune cells like neutrophils and macrophages may produce NGAL, an acknowledged acute phase reactant, which could increase the prevalence of AKI overall. More than that, NGAL (neutrophil gelatinase-associated lipocalin) is less likely to be removed from the body when the glomerular filtration rate (GFR) drops because of acute kidney injury (AKI); this causes NGAL to build up in the blood. It has been found that the dense ascending limb of the loop of Henle and the gathering ducts quickly increase the production of NGAL mRNA in people who have had acute kidney injury (AKI). As a result, NGAL protein is produced in the distal nephron (the renal pool) and then released into the urine. Most of the urine NGAL consists of this protein [11].

In group B, 39 patients (48.8%) had normal GFR and detectable NGAL levels (3.9 ng/ml-10 ng/ml), with mean urea levels of 19.2 mg/dl (±11.7 mg/dl), creatinine levels of 0.41 mg/dl (±0.11 mg/dl), and NGAL levels of 4.85 ng/ml (±0.8 ng/ml); this shows that NGAL levels rise before creatinine and GFR, making it a sensitive biomarker for acute renal damage.

Because creatinine measure’s function, not harm. It may not alter until 50% of renal function is gone. Additional confounding variables include age, gender, nutrition, muscle mass, race, and hydration state. Rises in NGAL levels indicate AKI 24–72 hours before diagnostic SCr rises and are prognostic.

[4]; this aligns with the conclusions of Dent et al., who evaluated a cohort of 120 neonates who had cardiopulmonary bypass (CPB) surgery for the management of congenital cardiac anomalies. In order to determine the progression of AKI, the NGAL and creatinine levels of each patient were evaluated. Serum creatinine levels were measured 2-3 days following the cardiopulmonary bypass (CPB), and AKI was seen in 45 patients (37%). However, after two hours of CPB, the average plasma NGAL levels increased fourfold and remained elevated throughout the whole trial period. Plasma NGAL had the highest level of predictive accuracy for AKI at two hours after CPB, with a statistically significant result ($P < 0.0001$) [11].

Acute kidney injury (AKI) was observed in 45 individuals, representing 75% of the total cases. After cardiopulmonary bypass, serum creatinine levels remained undetectable for 2-3 days. After 2 hours under constant CPB: positive pressure ventilation, the overall concentration of NGAL in blood plasma increased fourfold and remained elevated throughout the trial. NGAL: plasma neutrophil gelatinase-associated lipocalin levels were the best way to predict acute kidney injury (AKI) after two hours of cardiopulmonary bypass (CPB). They reached a significant level of $P < 0.0001$.

Fadel et al.'s research on 40 infants who underwent CPB operations for congenital cardiac conditions further supports this. Plasma NGAL and kidney function (creatinine, urea) were evaluated in all patients at 2, 12, and 24 hrs. post-surgery [12].

Plasma NGAL levels were significantly associated with serum creatinine & AKI at 2, 12, and 24 hrs. post-surgery ($p < 0.0001$), even though serum creatinine was not correlated with AKI up to 12 hours.

This experiment employed a receiver-operating characteristic (ROC) curve to assess the diagnostic efficacy of the NGAL biomarker. The 95% confidence interval (CI) spanned from 0.864 to 0.981, while the area under the curve (AUC) measured 0.940. The p-value is below 0.001. The accuracy was 88.4%, while the level of sensitivity was 88%. This study demonstrates that the NGAL biomarker is an accurate and dependable technique for the early detection of acute kidney damage (AKI).
This conclusion is consistent with the study conducted by Peco-Antić et al. involving 112 adolescents receiving CPB procedures. Acute kidney injury (AKI) was observed in 18 individuals, constituting 16.1% of the population. Plasma NGAL levels at 2–48 hours post-operation were significantly elevated in patients with AKI compared to those without AKI. NGAL had the highest AUC at 6 & 24 hours post-CPB (0.70 and 0.93) [13].

Conclusion

Serum creatinine is an inadequate biomarker for acute renal damage because it lacks specificity and has a sluggish response to changes in disease severity or therapy. Neutrophil gelatinase-associated lipocalin (NGAL) levels in plasma were checked in people with gastroenteritis and different levels of dehydration. It was shown that there is a direct correlation between the severity of dehydration and the concentration of NGAL. As the degree of dehydration worsens, the average value of NGAL rises.

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