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Neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C: potential biomarkers for early prediction of acute kidney injury in pediatric male patients

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Abstract

This study aims to estimate the relationship between Neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C and kidney function parameters and CRP in Acute Kidney Injury (AKI) patients. The instant research was designed to detect the level of serum markers in acute kidney injury patients in Kerbala City. 120 pediatric male samples participated, the present study was divided into two groups of 90 patients (31 Stage I,27 Stage II, and 32 Stage III) with acute kidney injury and 30 healthy control individuals matched for age and sex. The serum NGAL and Cystatin C levels were measured using an ELISA technique. Kidney function test (serum Creatinine and serum Urea, blood urea nitrogen (BUN), glomerular filtration rate (GFR), Electrolytes (Sodium (Na), potassium(K), and Chloride (Cl) and Urine output) were also measured, and Biochemical test (CRP and Albumin) were measured by quantitative method. The mean ±SD of age in (AKI) patients (5.94±4.93), while in healthy controls (7.70±5.02). The NGAL increased significantly in patients(5.69±1.23) when compared with control (2.30±0.28) and the Cystatin C increased significantly in patients (12.50±2.88) and healthy controls (5.32±1.71. The present study showed higher serum NGAL and Cystatin C in AKI patients related to early predicting renal complication and high levels of Kidney function tests (serum Creatinine, serum Urea, CRP, Chloride (Cl), and blood urea nitrogen (BUN)) in AKI and lower each of (glomerular filtration rate (GFR), Potassium (K), Albumin, and Urine output). Therefore, NGAL and Cys C can be considered a biomarker for Acute Kidney injury (AKI).

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INTRODUCTION

Acute kidney injury (AKI) is a rapid loss of kidney function that results from failing to maintain electrolyte homeostasis, acid-base balance, and fluid balance [1]. A sudden rise in blood creatinine, a drop in urine production, or both are indicative of acute kidney injury (AKI), this clinical syndrome is linked to a sudden decline in kidney function that can happen within hours to days and is frequently accompanied by oliguria [2]. AKI is a dangerous condition that affects pediatric and adolescents and can progress to chronic kidney disease and result in the need for dialysis if not diagnosed rapidly [3].

AKI is seen in approximately 5% of hospitalized patients, 27% of patients in the intensive care unit, and over 30% of adults following cardiac surgery have AKI; 3% of these patients require dialysis, and the fatality rate exceeds 70%. Acute kidney damage is still associated with high death rates, despite substantial technical breakthroughs in therapy. Even though urine volume and serum creatinine are frequently employed in primary care to assess AKI, they are not always accurate markers of acute changes in renal function. However, rather than kidney damage, urine volume, and serum creatinine are significant markers of renal function. As a result, alternative biomarkers are desperately needed to identify kidney damage [4] accurately.
NGAL, a member of the lipocalin superfamily, can accurately detect renal epithelial damage. NGAL is a single polypeptide protein secreted by activated neutrophils, with a molecular weight of 25 kDa. Initially, NGAL was present inside the granules of the neutrophils during the bone marrow maturation process. Lipocalin-2, a family of proteins involved in the transport of many chemicals, is another name for human NGAL [5], and has emerged as an early predictive biomarker for AKI. It has been found in tissue from the kidney, colon, and breast. Epithelial damage and neoplastic diseases are associated with higher levels of it. In kidney-related disorders, serum and urine levels are observed to be significantly elevated [6].
Cystatin C is a low molecular weight protein that every nucleated cell produces. and is present in relatively high amounts in various physiological fluids, most notably seminal fluid, cerebral fluid, and synovial fluid. Recently, serum cystatin C was proposed as a new endogenous marker of GFR. Cystatin C is a reliable biochemical marker with promising results worldwide [7]. This study aims to estimate the relationship between Neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C and some parameters in AKI patients

METHOD

Collection of data

A case-control observation study was carried out in the period from 2023 to 2024 at the kidney unit of Kerbala Teaching Hospital. The study group consisted of 30 healthy subjects and 90 pediatric male patients with AKI. A detailed physical examination of AKI patients was diagnosed by the clinic's neurologist, and the stages of AKI pediatrics were calculated by the RIFLE (risk, injury, failure, loss, end-stage, kidney disease) criteria [8], all participants with a range of age (1-20 years) from toddlers period (13 months) to late adolescence (21 years) according to the 2015 classification of pediatric patients by the National Institute of Child Health and Human Development [9] on average (5.94 ±4.93). A screening questionnaire was completed to gather data on age, height, weight, comorbidities, personal history, drug history, presenting problems, and laboratory investigations after obtaining informed consent.
The number of volunteers in the current study was divided into two groups as the following:
Group 1. Acute Kidney Injury (AKI) includes (90) patients (all males) divided into the following subdivisions:
A-Stage 1 (N=32).
B-Stage2 (N=27).
C-Stage 3 (N=31).
Group 2. Healthy control (HC) (N=30).
The patients were collected from September 2023 to February 2024. Unrelated, thirty healthy individuals corresponding to the patient's age and gender group were randomly selected as controls. Blood was collected from all members of the study groups to perform the biochemical tests. The concentrations of the serum NGAL and Cystatin C in patients and healthy controls were determined using an ELISA Kit, Serum creatinine, serum urea, CRP auto-determined by Cobas Integra 400 plus, and albumin were measured by (Colorimetric method) by (LINEAR CHEMICAL S.L).

Inclusion criteria

- Age from toddler period (13 months) to late adolescence (21 years).
- Any case with an acute increase in renal indices and decreased urine output.
- Only male patients.

Exclusion criteria

- Congenital anomaly of the renal system.
- Small size kidney.
- Any association with chronic diseases such as diabetes mellitus type 1, or congenital heart disease.
- Chronic renal failure
- Female patients.
- Patients that are more than 20 years old (adult).
- Patients that are less than 1 year old (Neonates and infants).

Ethical considerations

This study protocol was accepted after it was reviewed by a medical ethics committee at the University of Karbala College of Applied Medical. Also, the study achieves research ethics permission in Karbala Children's Teaching Hospital. The study objectives were described to all participants and verbal approvals were obtained from them. Sampling processing and laboratory biochemistry assay investigations for studied parameters were carried out in the laboratories of the previously mentioned hospitals.

Statistical Analysis:

Statistical Package of Social Science (SPSS) Version 24.0 was used to analyze the data, and the comparison, among groups was made by using analysis of variance (ANOVA table), A T-test was employed to assess the significance of arithmetic means the correlation between NGAL and Cystatin C level and other variables were assessed using sperman correlation. The statistically significant threshold was set at ($P \leq 0.01$).

RESULTS AND DISCUSSION

Table (1) shows age among AKI patients stages found highly significant differences ($P \leq 0.01$) between stage 3 and control and also between stage 1 and stage 3. Also, a non-significant difference in $P \geq 0.09$ between all patients and the control group, Body Mass Index (BMI) among AKI patients stages, and the control group found highly significant differences ($P \leq 0.001$) among all stages with control. Also, a high significant difference in $P \leq 0.001$ between all patients and the control group due to the proportion in this study will indicate that the majority of pediatrics with AKI suffer malnutrition and inactivity with a nutritional deficiency in comparison with pediatrics with normal or moderate nutritional deficiency and this agreement with [10], BMI was the most effective factor in predicting varying degrees of malnutrition[11] and disagreement with [12] that demonstrated high BMI increases the risk of AKI.

Table (1): The level of age and BMI in pediatric male patients with AKI stages compared to the control group

Variable	Groups	N	Mean	S.D.	P- value	LSD
Age(year)	control	30	7.70	5.02	0.01	2.48
	stage 1	32	7.73	5.13		
	stage 2	27	5.66	5.00		
	stage 3	31	4.31	4.03		
	All patients	90	5.94	4.93	0.09	6.96
BMI ($kg\backslash m^2$)	control	30	16.92	2.54	0.001	
	stage 1	32	14.75	1.83		
	stage 2	27	13.84	1.93		
	stage 3	31	14.67	1.87		
	All patients	90	14.45	1.90	0.001	

N=number of sample. S.D= standard deviation. LSD =least significant difference.

Table (2) showed creatinine among AKI patients stages and the control group found highly significant differences ($P \leq 0.001$) between stage 3 and control, stage 3 and stage 1, and stage 3 and stage 2. Also, a high significant difference in $P \leq 0.001$ between all patients and the control group related to that developed AKI, is defined as an increase in serum creatinine, and this is because of creatinine elimination by the kidney and when the kidney damage causes an increase in serum creatinine. Nevertheless, serum creatinine levels are used to diagnose and stratify AKI, which agrees with the [13] and urea among AKI patients stages and the control group found highly significant differences ($P \leq 0.001$) among stage 1, stage 2, and stage 3 with control, between stage 1 and stage 3, and between stage 2 and stage 3. Also, a high significant difference in $P \leq 0.001$ between all patients and the control group related to urea is it product of proteins and nitrogen metabolism, urea is the most abundant substance in the blood of uremic people [14] in patients with heart failure, decreased cardiac output and insufficient arterial filling lead to the release of the sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS), and increased sodium reabsorption in proximal renal tubules, resulting in increased urea concentration. Activation of SNS is a major cause of cardiac dysfunction and vascular injury and can significantly worsen the prognosis[15].

Urea is not age-related in the same way as creatinine but reflects fluid and protein intake as well as renal function. Volume depletion increases renal tubular uptake, causing increased serum urea, but in pediatrics, urine volume depletion is most often caused by less intake or gastrointestinal losses, and this agreement [8].

Table (2): The level of creatinine and urea in pediatric male patients with AKI stages compared to the control group

Variable	Groups	N	Mean	S.D.	P- value	LSD
Creatinine (mg\dl)	control	30	0.42	0.13	0.001	0.63
	stage 1	32	0.62	0.18		
	stage 2	27	0.97	0.36		
	stage 3	31	3.18	2.36		
	All patients	90	1.61	1.81	0.001	17.47
Urea (mg\dl)	control	30	20.61	5.13	0.001	
	-stage 1	32	61.10	17.99		
	-stage 2	27	74.85	24.42		
	-stage 3	31	174.79	62.30		
	All patients	90	104.38	65.27	0.001	

N=number of sample. S.D= standard deviation. LSD =least significant difference.

Table (3) shows Blood Urea Nitrogen (BUN) among AKI patients stages and the control group (3.21±0.80). Found highly significant differences (P≤0.001) among all stages with control and stage 1, stage 2 with stage 3. Also, a high significant difference in P≤0.001 between all patients and the control group related to Blood urea nitrogen (BUN), which is the standard measure of serum urea concentration, can be expressed as a molar concentration or even as a mass concentration. BUN can fluctuate rapidly enough during injury because people with normal renal function have a functional reserve that makes up for nephron damage [16] and this agrees with [17], and urine output among AKI patients stages and the control group found highly significant differences (P ≤0.001) among all stages with control and between stage 1, stage 2 with stage 3. Also, a high significant difference in P ≤0.001 between all patients and the control group related to developed AKI is defined as a decrease in urine output, and this is because AKI was pre-renal, renal, or post-renal summary by the variable pathophysiology of AKI, which is influenced by the various factors linked to its development. About 20% of the cardiac output, or a sizable portion of the total intravascular volume, is needed for renal perfusion. Once inside the kidney, it is dispersed specifically throughout the renal arteries. These arteries then branch out to form glomerular arterioles, which in turn form a network of capillaries that perform glomerular filtration—the process of filtering waste products and other molecules like proteins. Renal blood flow under normal circumstances is between 5 and 6 ml/g/min at a pressure between 60 and 100 mmHg, which is required to preserve renal function [13]. Vascular tone, neurohormonal processes, and vasodilators are among the several extrarenal processes that largely control renal blood flow[2].
However, urine output decrease alone is insufficient for the diagnosis of AKI, and the sensitivity and specificity of urine output decrease are insufficient to make a diagnosis, so the diagnosis and classification of AKI are done using serum creatinine levels and urine output. Furthermore, it's important to note that a healthy renal functional reserve can lessen the rise in serum creatinine and this agrees with [18] demonstrates that developed AKI, defined as an increase in serum creatinine (sCr) or decrease in urine output.

Table (3): level of BUN, urine output, and GFR in pediatric male patients with AKI stages compared to the control group

Variable	Groups	N	Mean	S.D.	P- value	LSD
BUN (mg\dl)	control	30	3.21	0.80	0.001	2.84
	stage 1	32	9.50	2.80		
	stage 2	27	11.64	3.80		
	stage 3	31	27.19	9.69		
	All patients	90	16.24	10.15	0.001	
urine output (ml\kg\hr)	control	30	1.56	0.46	0.001	0.12
	-stage 1	32	0.39	0.06		
	-stage 2	27	0.38	0.05		
	-stage 3	31	0.14	0.09		
	All patients	90	0.30	0.13	0.001	

N=number of sample. S.D= standard deviation. LSD =least significant difference.

This study used three formulas to estimate Glomerular Function Rate (GFR) and compare among them as shown in Figure (1) and demonstrated a highly significant reduction in GFR in AKI patient groups compared to the control groups in each of the formulas related to Renal blood flow is essentially controlled by a variety of extrarenal processes, including chemicals that function as vasodilators or vasoconstrictor, neurohormonal processes, and vascular tone. Therefore, if any of these mechanisms fail, hypoxia will result, which will reduce blood flow and glomerular filtration to generate the appropriate amount of urine. Instead of being a single illness, acute kidney injury is a loose syndrome made up of several ailments such as sepsis, cardiovascular issues, nephrotoxicity, urinary tract blockage, and anything that can cause glomerular filtration rate (GFR) to be reduced this agreement with [19] quickly.

The result of GFR by SCr & Cys C formula was detected as a decrease among AKI patients than in control groups as shown in Table (4). The analytical study demonstrated highly significant variations at ($P \leq 0.0001$) among all stages with control and between stage 2 and stage 3 with stage 1 and between stage 2 and stage 3.

Table (4): The GFR by SCr and Cys C of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P- value	LSD
GFR by SCr and Cystatin C (ml\min\1.73m^2)	Control	30	50.30	7.73	0.0001	2.71
	stage 1	32	28.56	4.71		
	stage 2	27	20.74	2.99		
	stage 3	31	12.48	4.07		
	All patient	90	20.68	7.85	0.0001	

N=number of sample. S.D= standard deviation. LSD =least significant difference.

The result of GFR by SCr formula was detected as a decrease among AKI patients in each stage of AKI than in control groups as shown in Table (5). The analytical study demonstrated highly significant variations at ($P \leq 0.0001$) between all stages with control and between stages 2 and 3 with stage 1 and between stage 2 and stage 3.

Table (5): The GFR by SCr of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S. D.	P-value	LSD
GFR by SCr (ml\min\1.73m2)	Control	30	109.71	13.06	0.0001	4.70
	stage 1	32	81.40	4.08		
	stage 2	27	48.25	8.10		
	stage 3	31	17.94	8.80		
	All patient	90	49.60	27.64	0.0001	

N=number of sample. S.D= standard deviation. LSD least significant difference.

The result of GFR by the Cys C formula was detected as a decrease among AKI patients in each stage of AKI than in control groups as shown in Table (6). The analytical study demonstrated highly significant variations at ($P \leq 0.0001$) between all stages with control.

Table (6): The GFR by Cys C of pediatric male patients with AKI patients as compared to the control group

Variable	Groups	N	Mean	S.D.	P-value	LSD
GFR by Cystatin C ((ml\min\1.73m2)	Control	30	16.30	5.07	0.0001	1.84
	stage 1	32	7.41	2.84		
	stage 2	27	6.70	2.16		
	stage 3	31	7.77	3.32		
	All patient	90	7.32	2.84	0.0001	

N=number of sample. S.D= standard deviation. LSD =least significant differences

The comparison among these formulas shown in Table (7) demonstrated that the Cystatin C formula more accurate in estimating GFR related to creatinine is inaccurate at detecting mild renal impairment, and creatinine levels can vary with muscle mass but little with protein intake whenever cystatin C might provide more accurate information compared to serum creatinine because it is unaffected by sex, age and this agreement with [2], related to demonstrates three formulas to estimate GFR in patient groups and control groups and compares these three formulas. The first is performed by using SCr which is nearly entirely filtered by the glomerular and reabsorbed in the proximal tubule and the second by using cystatin C another element that is filtered by the glomerular and almost completely reabsorbed in the proximal tubule that constant production and renal elimination make it an excellent biomarker of glomerular filtration and this agrees with [13], and the third formula by using SCr and Cystatin C formula [20].

Table (7): The level of GFR in pediatric male patients with AKI stages compared depends on three equations.

GFR	Mean	S. D	P-value	LSD
GFR by SCr and Cystatin C (ml\min\1.73m2)	20.68	7.85	0.001	4.87
GFR by Cystatin C (ml\min\1.73m2)	7.32	2.84		
GFR (ml\min\1.73m2) by SCr	49.60	27.64		

S.D= standard deviation. LSD =least significant difference.

Table (8) shows sodium among AKI patients stages and the control group found non-significant differences (P>0.01) among all stages with control and between each stage. Also, a nonsignificant difference in P>0.63 between all patients and the control group this result is in agreement with [21] who found a high increase in Na electrolyte in AKI patients related to variations in the GFR and the salt supply to the renal tubules, which cause an imbalance between the supply and demand of oxygen nutrients, damaging tubular epithelial cells, and result in oxidative stress [13] and disagrees with [22] that demonstrated that electrolyte disorders are associated with worse outcomes, with increased hospitalization length and mortality, and potassium among AKI patients stages and the control group found high significant differences (P≤0.001) between stage 2 and stage 3 with control and between stage 1 and stage 3. Also, a significant difference in P≤0.01 between all patients and the control group related to Many complications, including metabolic acidosis, and elevated blood potassium levels with decreased excretion [23], this result disagrees with [22] that found hypokalemia and demonstrated that by this; electrolyte disorders are associated with worse outcomes, with increased hospitalization length and mortality, and Cl among AKI patients stages and the control group. Found significant differences (P≥0.05) between stage 1 and control. Also, a significant difference in P≥0.03 between all patients and the control group in the study shows an increase of chloride (Cl) related to the compromised ability to concentrate or dilute urine, In AKI, chloride levels may be elevated or decreased, depending on the underlying cause and severity of the injury. Elevated chloride levels may be associated with dehydration, and this agreement [24].

Table (8): level of Sodium, potassium, and chloride in pediatric male patients with AKI stages compared to control group

Variable	Groups	N	Mean	S.D.	P- value	LSD
Na (mmol\l)	control	30	137.17	3.61	0.12	n. s
	stage 1	32	140.80	8.32		
	stage 2	27	138.24	11.85		
	stage 3	31	135.26	11.14		
	All patients	90	138.12	10.60	0.63	
K (mmol\l)	control	30	4.25	0.45	0.001	.54
	stage 1	32	4.47	0.92		
	stage 2	27	4.96	0.99		
	stage 3	31	5.16	1.61		
	All patients	90	4.85	1.25	0.01	
Cl (mmol\l)	control	30	103.26	2.81	0.05	5.77
	stage 1	32	111.11	9.80		
	stage 2	27	107.31	13.65		
	stage 3	31	106.32	14.41		
	All patients	90	108.32	12.74	0.03	

N=number of sample. S.D= standard deviation. LSD least significant differences

Table (9) albumin among AKI patients' stages and the control group found highly significant differences (P≤0.001) among all stages with control. Also, a high significant difference in P≤0.001 between all patients and the control group related to malnutrition in patients with AKI that causes hypoalbuminemia and related to the effect of such other factors as proteinuria, a common problem in patients with renal failure. The specificity of albumin level as a nutritional marker decreases in case of inflammation and fluid overload and acidemia also affects serum albumin levels that agree with [11-12], and CRP among AKI patients' stages and the control group (2.60±0.84). found highly significant differences (P≤0.001) in all stages with control. Also, a high significant difference in P≤0.001 between all patients and the control group related to AKI as the spread of inflammatory processes from the kidney to other organ systems is affected by metabolic changes and a direct and significant relationship between inflammation with CRP level [11], Therefore, malnutrition patients are more exhibition to AKI and this agreement with [12].

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Table (9): The level of albumin and CRP in pediatric male patients with AKI stages compared to the control group

Variable	Groups	N	Mean	S.D.	P- value	LSD
Albumin (g\dl)	control	30	4.25	0.56	0.001	0.43
	stage 1	32	2.64	0.98		
	stage 2	27	2.57	0.89		
	stage 3	31	2.91	0.91		
	All patients	90	2.71	0.93	0.001	
CRP (mg\dl)	control	30	2.60	0.84	0.001	24.34
	stage 1	32	33.93	64.75		
	stage 2	27	35.81	29.45		
	stage 3	31	54.29	64.41		
	All patients	90	41.51	56.56	0.001	

N=number of sample. S.D= standard deviation. LSD= least significant differences.

Serum level of NGAL

Table (10) shows NGAL among AKI patients' stages 1 and the control group found highly significant differences (P≤0.01) among all stages with control. Also, a high significant difference in P≤0.01 between all patients and the control group. NGAL was first identified in neutrophil granules; it is nearly entirely reabsorbed in the proximal tubule, and increased levels can be a sign of proximal tubular damage [25]. This pro-inflammatory mediator is generated as a result of tissue damage and acts as a biomarker for the early detection of kidney injury. All nephron segments have the potential to be harmed after an ischemia event, however the proximal tubular cells are typically the most injured. When AKI is present, the distal tubule and Henle's loop can produce 1000 times more NGAL, and this agrees with [26] and disagrees with [27], which demonstrated that NGAL showed a slight increase in their study of kidney injury.

Table (10): The NGAL in pediatric male patients with AKI stages compared to the control group

Variable	Groups	N	Mean	S.D.	P- value	LSD
NGAL	control	30	2.30	0.28	0.001	0.56
	stage 1	32	5.51	1.23		
	stage 2	27	5.84	1.08		
	stage 3	31	5.75	1.36		
	All patient	90	5.69	1.23	0.001	

N=number of sample. S.D= standard deviation. LSD =least significant differences.

Table (11) showed a positive correlation (r=0.456) existed between NGAL and urea that was statistically significant at (P ≤0.01), a positive correlation (r=0.231) existed between NGAL and creatinine that was statistically significant at (P ≤0.01), a negative correlation (r= -0.508) existed between NGAL and albumin that was statistically significant at (P ≤0.01), a positive correlation (r=0.455) existed between NGAL and BUN that was statistically significant at (P ≤0.01), and showed a negative correlation (r= -0.727) existed between NGAL and urine output that was statistically significant at (P ≤0.01).

Table (11): Correlation of NGAL biomarkers in the patient group.

	NGAL (ng\ml)	Urea (mg\dl)	creatinine (mg\dl)	Albumin (g\dl)	BUN(mg\ dl)
Urea (mg\dl)	.456**				
creatinine (mg\dl)	.231*	.754**			
Albumin (g\dl)	-.508**	-.336**	-.109		
BUN(mg\dl)	.455**	1.000**	.754**	-.336**	
urine output(ml\kg\hr)	-.727**	-.622**	-.423**	.496**	-.622**

**. Correlation: significant at the 0.01 level (2-tailed).*. Correlation: significant at the 0.05 level (2-tailed).

According to Table (12), the findings showed a positive correlation (r=0.296) existed between NGAL and K that was statistically significant at (P ≤0.01), a positive correlation (r=0.189) existed between NGAL and Cl that was statistically significant at (P <0.05), and a positive correlation (r=0.835) existed between Na and Cl that was statistically significant at (P ≤0.01).

Table (12): Correlation of NGAL biomarkers in the patient group.

Parameters	NGAL (ng\ml)	Na (mmol\l)	k (mmol\l)
Na (mmol\l)	.030		
k (mmol\l)	.296**	.124	
Cl (mmol\l)	.189*	.835**	.172

**. Correlation: significant at the 0.01 level (2-tailed).*. Correlation: significant at the 0.05 level (2-tailed).

According to Table (13) the findings showed a negative correlation (r=-0.190) existed between NGAL and weight that was statistically significant at (P <0.05), a negative correlation (r= -0.391) existed between NGAL and BMI that was statistically significant at (P ≤0.01), a negative correlation (r= -0.608) existed between NGAL and GFR by SCr that was statistically significant at (P ≤0.01), a negative correlation (r= -0.771) existed between NGAL and GFR by SCr & Cys C that was statistically significant at (P ≤0.01) ,and a negative correlation (r= -0.850**) existed between NGAL and Cys C that was statistically significant at (P ≤0.01).

Table (13): Correlation of NGAL biomarkers in the patient group.

Parameters	NGAL (ng\ml)	Cystatin C (ng\ml)	Age (year)	Weight (Kg)	Height (m)	BMI (kg\m2)	GFR (ml\min\1.73m2) by SCr
Cystatin C (ng\ml)	.850**						
Age(year)	-0.119	-0.038					
Weight (Kg)	-.190*	-0.127	.959**				
Height (m)	-0.119	-0.011	.956**	.918**			
BMI (kg\m2)	-.391**	-.452**	.401**	.572**	.314**		
GFR by SCr(ml\min\1.73m2)	-.608**	-.522**	.298**	.372**	.313**	.373**	
GFR by SCr and Cystatin C(ml\min\1.73m2)	-.771**	-.761**	.305**	.392**	.305**	.472**	.918**

**. Correlation: significant at the 0.01 level (2-tailed).*. Correlation: significant at the 0.05 level (2-tailed).

There is a direct correlation between NGAL and CRP related to that each of them related to inflammation [28-29]. The positive correlation between NGAL, creatinine, urea, and BUN related to the increase in each of them related to kidney injury, NGAL is utilized as a kidney damage biomarker since it can be secreted by dysfunctional tubular cells and transported into the serum., Unlike BUN and Scr, NGAL is not a direct indicator of renal function. To evaluate the diagnostic ability of NGAL with BUN and Scr, we continued to use them as references in the current investigation[30].

The positive correlation between NGAL, Na, and K that related to each increase in kidney injury. An increase in sodium that is linked to variations in GFR and the supply of sodium (Na) to the renal tubules causes an imbalance between oxygen nutrition availability and demand, harming tubular epithelial cells and leading to oxidative stress [21], increasing potassium related to many complications that can result from AKI, including metabolic acidosis, and elevated blood potassium levels with decreased excretion [23], and an increase of NGAL was discovered in the granules of neutrophils.; it is virtually entirely reabsorbed in the proximal tubule, and excessive levels may be a sign of injury to the proximal tubule [25]. There is a positive correlation between NGAL and Cys C related to the increase of related to kidney injury[31], while the negative correlation between NGAL, BMI, weight, and GFR was related to increased NGAL and decreased BMI, weight, and GFR.

Serum Level of Cystatin C

Table (14) shows Cystatin C among AKI patients stages and the control group found highly significant differences (P≤0.01) among all stages with control and between stage 2 and stage 3. Also, a significant difference in P-value = 0.001 between all patients and the control group related to all nucleated cells studied to date produce cystatin C at a consistent rate, which is then readily reabsorbed in the proximal tubule almost totally after being filtered by the glomeruli. It is expressed at low levels in the kidney and other organs in healthy kidneys, but because of a lower glomerular filtration rate in renal damage, its expression is more prominent. This observation is consistent with [13-2] and disagreement with [26] who demonstrated a decrease of cystatin C in kidney injury in their study.

Table (14): The Cys C in pediatric male patients with AKI stages compared to the control group

Variable	Groups	N	Mean	S.D.	P- value	LSD
Cystatin C	control	30	5.32	1.71	.00	1.36
	stage 1	32	12.52	3.02		
	stage 2	27	13.23	2.42		
	stage 3	31	11.85	3.04		
	All patient	90	12.50	2.88	.00	

N=number of sample. S.D= standard deviation. LSD =least significant

Table (15) a positive correlation (r=0.366) existed between Cys C and urea that was statistically significant at (P ≤0.01), a positive correlation (r=0.214) existed between Cys C and creatinine that was statistically significant at (P<0.05), a negative correlation (r= -0.453) existed between Cys C and albumin that was statistically significant at (P ≤0.01), a positive correlation (r=0.366) existed between Cys C and BUN that was statistically significant at (P ≤0.01) and showed a negative correlation (r= -0.675) existed between Cys C and urine output that was statistically significant at (P ≤0.01).

Table (15): correlation of cystatin C biomarker in the patient group.

Parameters	Cystatin C (ng\ml)	Urea (mg\dl)	creatinine (mg\dl)	Albumin (g\dl)	BUN (mg\dl)
Urea (mg\dl)	.366**				
creatinine (mg\dl)	.214*	.754**			
Albumin (g\dl)	-.453**	-.336**	-.109		
BUN (mg\dl)	.366**	1.000**	.754**	-.336**	
urine output(ml\kg\hr)	-.675**	-.622**	-.423**	.496**	-.622**

**. Correlation: significant at the 0.01 level (2-tailed).*. Correlation: significant at the 0.05 level (2-tailed).

Table (16) the findings showed a negative correlation (r= -0.452) existed between Cys C and BMI that was statistically significant at (P ≤0.01), a negative correlation (r= -0.522) existed between Cys C and GFR by SCr that was statistically significant at (P ≤0.01), a negative correlation (r= -0.761) existed between Cys C and GFR by SCr & Cys C that was statistically significant at (P ≤0.01), and a negative correlation (r= -0.922) existed between Cys C and GFR by Cys C that was statistically significant at (P ≤0.01).

Table (16): correlation of cystatin C biomarker in the patient group.

Parameters	Cystatin C(ng\ml)	Age(year)	Weight (Kg)	Height (m)	BMI (kg\m2)	GFR (ml\min\1.73m2) by SCr	GFR by SCr and Cystatin C(ml\min\1.73m2)
Age(year)	-.039						
Weight (Kg)	-0.127	.959**					
Height (m)	-.011	.956**	.918**				
BMI (kg\m2)	-.452**	.401**	.572**	.314**			
GFRby SCr(ml\min\1.73m2)	-.522**	.298**	.372**	.313**	.373**		
GFR by SCr and Cystatin C(ml\min\1.73m2)	-.761**	.305**	.392**	.305**	.472**	.918**	

There is a positive correlation between Cys C and CRP related to each of them connected to inflammation. It was recently found that there is a strong correlation between CRP levels and serum cystatin C content[32]. The positive correlation between Cys C and creatinine, urea, and BUN is related to that each of them Cys C is a glomerular filtration biomarker Cys C levels rise earlier than urea and creatinine when the kidney is injured [32], while the negative correlation between albumin and urine output is related to a decrease each of them in kidney injury. A negative correlation between Cys C, BMI, and GFR is related to an increase in Cys C, which is considered an earlier biomarker for the diagnosis of AKI [32], while a decrease in each BMI due to malnutrition accompanied by kidney injury and a decrease in GFR. According to a recent study by [33], estimates of eGFR that take into account both creatinine and cystatin C are more accurate than those that only consider creatinine or cystatin C. Many factors, including total muscle mass, recent muscle damage, GFR, and protein intake, can have a significant impact on the SCr level [34]. Age and ethnicity had less of an impact on eGFR based on cystatin C; nevertheless, serum levels can be impacted by smoking, obesity, inflammation, and glucocorticoid consumption [35].

CONCLUSION

We can conclude from the present study the serum concentration of NGAL and Cys C was significantly higher in all patient groups than in healthy control and it was highly significantly different between acute kidney injury and healthy groups, this indicates the role of this biomarker in the early identification and prediction of AKI-related complications and may facilitate supportive medical care for positive patient outcomes. There is a significant difference that could be seen in NGAL and Cys C between the patient group and control group and between stages groups.

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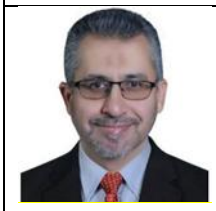

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