

ORIGINAL PAPER

Evaluation of the levels of kidney injury molecule-1 and cystatin C as early biomarkers for prediction of acute kidney injury complications in paediatric male patients – a case-control study

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ABSTRACT

Introduction: Acute kidney injury (AKI) is a serious problem in children and adolescents because it is associated with higher morbidity and mortality, and it may progress rapidly and lead to chronic renal disease, which would require dialysis. Kidney injury molecule-1 (KIM-1) and serum cystatin C are recognised as early sensitive and specific biomarkers for kidney injury. The study aims to predict KIM-1, cystatin C, and other kidney function biomarkers in AKI paediatric patients.

Material and methods: A total of 120 paediatric males were used in the present study divided into 90 patients with AKI with a mean age of 5.94 ± 4.93 years. All patients were divided according to RIFLE (renal, injury, failure loss, end- stage) for 3 stages: stage 1 (32 patients); stage 2 (27 patients); and stage 3 (31 patients), and controls (30) with a mean age of 7.70 ± 5.02 years. The data were collected for this research at the Kidney Unit in Kerbala Teaching Hospital, Kerbala, Iraq.

Results: The study found that KIM-1 significantly ($p \leq 0.001$) increased in all stages of AKI patients: stage 1 (1501.64 ± 417.02); stage 2 (1638.91 ± 354.85); stage 3 (1474.16 ± 425.63), and the controls (716.64 ± 50.72); moreover, highly significant differences ($p \leq 0.001$) in cystatin C in all stages of AKI patients: stage 1 (12.52 ± 3.02); stage 2 (13.23 ± 2.42); stage 3 (11.85 ± 3.04); and the controls (5.32 ± 1.71). The glomerular filtration rate (GFR) by s Cr/cystatin C ratio was significantly ($p \leq 0.001$) decreased in all stages. The highest decrease of GFR was calculated for cystatin C for all stages compared to the control and highly significant differences ($p \leq 0.001$) in elevated levels of kidney function tests (serum creatinine, serum urea, C-reactive protein – CRP, chloride – Cl, potassium – K, and blood urea nitrogen – BUN) in AKI and highly significant differences ($p \leq 0.001$) in the decrease of GFR, albumin, and urine output.

Conclusions: Finally, higher serum KIM-1, serum cystatin C, serum creatinine, serum urea, CRP, Cl, K, and BUN, while decreasing each of the GFR, albumin, and urine output in AKI patients, are related to early prediction of renal complications. Therefore, KIM-1 and cystatin C can be considered as biomarkers for AKI.

KEY WORDS:

acute kidney injury (AKI), cystatin C, kidney injury molecule-1 (KIM-1), blood urea nitrogen (BUN), glomerular filtration rate (GFR).

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INTRODUCTION

Acute kidney injury (AKI) is one of the most important global public health issues. Many complications can lead to several consequences, including metabolic acidosis, increased potassium (K) levels in the blood, uraemia, and fluid balance abnormalities. Heart failure, stroke, and cardiovascular disease are other long-term consequences of AKI. Most paediatric AKI deaths are caused by infections and cardiovascular conditions [1].

The hallmark of AKI is a sudden decrease in the kidney's capacity to filter waste materials, which increases the levels of waste materials like urea and creatinine in the bloodstream with or without changes in urine production [2].

Acute kidney injury occurs in 13–78% of ill patients [3]. Acute kidney injury causes approximately 2 million deaths worldwide each year [4], a high risk of AKI-associated death (13.8%), and a global incidence of juvenile AKI of 33.7%.

Kidney injury molecule-1 (KIM-1), also called T-cell immunoglobulin mucin-1 (TIM-1), type 1 transmembrane glycoprotein KIM-1, with a molecular weight of 104 da, is mostly expressed on T-cell surfaces and causes 2 extracellular kidney injuries in proximal tubular cells and hepatitis A virus cellular receptor 1 in hepatocytes [5].

The extracellular domain of KIM-1 is cleaved by matrix metalloproteinase enzyme and is present in the urine and blood of humans after proximal tubular injury. Studies show that KIM-1 functions as a prognostic indication as well as a specific and sensitive marker of kidney injury. The usefulness of serum KIM-1, an ambiguous early indicator of renal impairment, has not been thoroughly studied. Nonetheless, numerous investigations have shown that KIM-1 is a precursor to AKI or chronic kidney disease (CKD) [6].

Because KIM-1 can diagnose AKI sooner, it is thought to be the most viable protein among blood and urine indicators. Furthermore, KIM-1 can represent the injury and recovery processes in an intrusive manner. The cause is because KIM-1's ectodomain cleaves and is found in both blood and urine [7]. A recent study showed that serum creatinine increased after 48 hours, although urine and serum KIM-1 increased after 12 hours [8].

Additionally, KIM-1 is a phosphatidylserine receptor located on renal epithelial cells that identifies and facilitates the phagocytosis of apoptotic cells [9]. It is expressed in low levels in the kidney and other organs, but its expression is accentuated in pre-renal kidney injury and after reperfusion [10].

Kidney injury molecule-1 is an adhesion molecule observed in mucous cells. It plays a central role in the procedure of mucous regeneration and removing dead cells from the tubular lumen through phagocytosis. A small amount of KIM-1 and its messenger ribonucleic acid is acceptable in a normal kidney; however, because KIM-1 is

present in the proximal tubular epithelium, and the proximal tubular epithelium is susceptible to injury due to ischaemia [11]. Significant increases in their concentration can be regarded as a sign of an ischaemic kidney [12].

Kidney injury molecule-1, also identified as TIM-1, was found to be the most upregulated protein in the proximal renal tubule after a wide variety of injurious influences including ischaemia, nephrotoxic agents, sepsis, and immune-related injury, and its cleaved ectodomain is often used as a blood and urine marker for kidney injury [13].

A low-molecular-weight protein, cystatin C, is produced by every nucleated cell and is present in relatively high amounts in various physiological fluids, most notably seminal fluid, cerebral fluid, and synovial fluid [9].

Cystatin C is a cysteine-protease inhibitor molecular weight of 13.3 kDa that is freely filtered but is degraded and/or resorbed in the renal tubule so that none appears in the urine. Serum cystatin C is a well-known, clinically applicable biochemical parameter for the assessment of acute kidney injury. Serum cystatin C has been proposed as an ideal marker for assessing glomerular filtration rate (GFR). Several studies have shown that serum cystatin C is a more sensitive indicator of an early and mild reduction in renal functions than serum creatinine (sCr) [14].

Recently, cystatin C has been shown as a useful biomarker in AKI prediction; its urinary excretion indicates tubular damage, and in contrast to creatinine it has a moderate diagnostic utility, its concentrations are increased in both acute and chronic kidney disease, and it is not influenced by inflammatory processes, height, weight, age, sex, or nutritional condition. Cystatin C is filtered in the glomeruli and is completely resorbed and degraded in the proximal tubules, not secreted in the tubules. Therefore, it does not pass into the bloodstream, and this makes it a suitable endogenous marker for GFR estimation. In contrast to creatinine, cystatin C is not affected by age, sex, muscle mass, body composition, inflammatory processes, or diet [10]. Its level rises earlier (12–24 hours) than that of serum creatinine [15].

In the absence of renal disease, the serum concentration of cystatin C is increased in patients with liver illness, thyroid disease, and during glucocorticoid treatment. Because it is broken down in the kidneys, it is not found in the urine, and the calculation of renal clearance is not possible [16].

Early detection of AKI biomarkers can significantly reduce renal damage, improve the results in the long term, and prevent the progression of CKD. These biomarkers could also help identify patients who can benefit from early intervention before the AKI becomes clinically detectable. Early diagnosis of renal disease complication or regeneration is essential for the prognosis of patients as well as to reduce medical costs and time. Therefore, the level of KIM-1, cystatin C, and other kidney function biomarkers in AKI paediatric patients can be used

to predict renal complications and prevent CKD in such patients.

MATERIAL AND METHODS

A case-control observation study was carried out in the period September 2023 – February 2024 at the kidney unit of Kerbala Teaching Hospital. In the study group, 30 healthy paediatric males corresponding to the patient's age were randomly selected as controls with no history of kidney disease or any significant comorbidities, and 90 paediatric male patients with AKI in different stages of AKI 32 (stage 1), 27 (stage 2), and 31 (stage 3) were selected. A detailed physical examination of AKI patients was carried out by the clinic's neurologist, and the stages of AKI paediatrics were calculated by the risk, injury, failure, loss, end-stage, kidney disease criteria [17] in all participants with a range of age (1–20 years) from toddlers (13 months) to those in late adolescence (21 years) according to the 2015 classification of paediatric patients by the National Institute of Child Health and Human Development [18] – on average 5.94 ± 4.93 . After obtaining informed consent, a screening questionnaire was completed to gather data on age, height, weight, comorbidities, personal history, drug history, presenting problems, and laboratory investigations.

INCLUSION CRITERIA

Age from toddler period (one year) to late adolescence (20 years), any case with an acute increase in renal indices and decreased urine output, and only male patients were regarded as inclusion criteria.

EXCLUSION CRITERIA

Congenital renal system anomaly, small size kidney, any association with chronic diseases (such as diabetes mellitus type 1, or congenital heart disease), chronic renal failure, female patients, and patients' age over 21 years.

SAMPLE COLLECTION AND ANALYSIS

Blood samples were obtained concurrently and examined. They were taken within 24 hours of the injury's start in the AKI study group by withdrawing 5 ml of venous blood samples and then placed in disposable plain tubes. They were then allowed to clot at room temperature and centrifuged at 3000 rpm for 15 min. Serum was separated and stored in a plain tube at -20°C and then used for measuring creatinine, urea, albumin, CRP, and other parameters, which were auto-determined using a Cobas Integra 400 Plus, Germany. The serum KIM-1 and cystatin C concentrations in patients and healthy controls were determined using an ELISA Kit Cloud clone, USA. The study included measuring biochemical markers such

as albumin and CRP, creatinine, and urea, by using an automated chemistry analyser by Cobas Integra 400 Plus, Germany, GFR by 3 different formulas, as well as blood urea nitrogen (BUN) and urine output with special formula electrolytes (sodium – Na, K, and chloride – Cl) using an EX-DS Electrolyte analyser, JOKOH, Japan, at the Kerbala Teaching Hospital. Five millilitres of blood were collected from all members of the study groups to perform the biochemical tests. The concentrations of the serum KIM-1 in patients and healthy controls were determined using an ELISA Kit. Serum creatinine, serum urea, and CRP were auto-determined by Cobas Integra 400 plus, and albumin was measured by colorimetric method (Linear Chemical S.L.).

In the patient's urine output record, it is important to note when the catheter bag or chamber was last emptied, examine it at eye level, and record the amount of urine present, usually measured in millilitres. One should also record the volume of urine on the appropriate urine output chart along with the estimation time. Finally, if the catheter bag is full, it should be emptied after recording.

ESTIMATION OF URINE OUTPUT

Divide the volume of urine generated by the number of hours since the bag or chamber was last emptied to determine the rate of urine output. A healthy person's normal urine production should be between 0.5–1.5 ml/kg/hour, and patients should typically be peeing every six hours. The inability to produce enough pee (less than 0.5 ml/kg/h in children) is known as oliguria.

Urine output = volume of urine/number of hours [19].

ESTIMATION OF GLOMERULAR FILTRATION RATE

1. Creatinine-based "Bedside Schwartz" equation: $\text{eGFR} = 0.413 \times (\text{height in cm}) \div \text{serum Cr}$ [20, 21]
2. Cystatin C-based equation: $\text{eGFR} = 70.69 \times (\text{Cys C})^{-0.931}$ [20, 21]
3. Creatinine-cystatin C-based equation: $\text{eGFR} = 39.8 \times [\text{height}/\text{sCr}]^{0.456} \times [1.8/\text{Cys C}]^{0.418} \times [30/\text{BUN}]^{0.079} \times [1.076 \text{ male}] \times [\text{height}/1.4]^{0.179}$ [20, 21]

ESTIMATION OF BODY MASS INDEX

Weight by kg, and height by metre → calculate body mass index (BMI): $\text{BMI} = \text{weight (kg)}/\text{height (m)}^2$ [22, 23]

Estimation of blood urea nitrogen

$\text{BUN (mg/dl)} = \text{urea (mmol/l)} \times 2.8$ [24].

STATISTICAL ANALYSIS

Statistical Package of Social Science Version 24.0 was used to analyse the data. The comparison among groups was made by using analysis of variance (ANOVA table), Student's *t*-test was employed to assess the significance

of arithmetic means. The correlation between KIM-1 and cystatin C level and other variables was assessed using Spearman correlation. The statistically significant threshold was set at $p \leq 0.01$.

RESULTS

Figure 1 demonstrated a highly significant difference ($p \leq 0.001$) in age between stage 3 of AKI (4.31 ± 4.03) and controls (7.70 ± 5.02) and stage 1 (7.73 ± 5.13), while no significant difference between stage 1 (7.73 ± 5.13) and stage 2 (5.66 ± 5.00) with controls and stage 1 and stage 3 with stage 2.

The results in Table 1 demonstrate highly significant differences ($p \leq 0.001$) in weight between stage 2 (18.65 ± 12.65) and stage 3 (15.33 ± 10.10) with controls (27.85 ± 16.80) and between stage 1 (25.33 ± 15.30) and stage 3, while no significant difference between stage 1 with controls, and between stage 2 with stage 1 and stage 3.

The results in Table 1 demonstrate highly significant differences ($p \leq 0.01$) in height between stage 3 (0.98 ± 0.29) and controls (1.22 ± 0.32) and among stage 1 (1.24 ± 0.35) with stage 2 (1.06 ± 0.38) and stage 3. There is no significant difference between stage 1, stage 2 with control, and stage 2 with stage 3.

Figure 2 demonstrates a highly significant difference ($p \leq 0.001$) in BMI among stage 1 (14.75 ± 1.83), stage 2 (13.84 ± 1.93), and stage 3 (14.67 ± 1.87) with control (16.92 ± 2.54), while nonsignificant difference between stage 1, stage 2, and stage 3 was found.

Table 2 demonstrates a highly significant difference ($p \leq 0.001$) in creatinine between stage 3 (3.18 ± 2.36) and controls (0.42 ± 0.13), stage 3 and stage 1 (0.62 ± 0.18), and stage 3 and stage 2 (0.97 ± 0.36), while nonsignificant difference between stage 1, stage 2 with controls, and stage 1 with stage 2.

A highly significant difference ($p \leq 0.001$) in urea was demonstrated between stage 1 (61.10 ± 17.99), stage 2 (74.85 ± 24.42), and stage 3 (174.79 ± 62.30) with controls (20.61 ± 5.13), between stage 1 and stage 3, and between stage 2 and stage 3, while a nonsignificant difference was shown between stage 1 and stage 2.

A highly significant difference ($p \leq 0.001$) was shown in BUN among all stages with control and stage 1 (9.50 ± 2.80), stage 2 (27.19 ± 9.69) with stage 3 (11.64 ± 3.80),

while nonsignificant difference was shown between stage 1 and stage 2.

A highly significant difference ($p \leq 0.001$) in urine output was shown between stage 1 (0.39 ± 0.06), stage 2 (0.38 ± 0.05), stage 3 (0.14 ± 0.09) and the control group (1.56 ± 0.46) and between stage 1, stage 2, with stage 3, while no significant difference was shown between stage 1 and stage 2.

A highly significant difference ($p \leq 0.001$) was shown in albumin among stage 1 (2.64 ± 0.98), stage 2 (2.57 ± 0.89), and stage 3 (2.91 ± 0.91) with the control group (4.25 ± 0.56), while no significant difference between each stage was shown.

A highly significant difference ($p \leq 0.001$) in CRP between stage 1 (33.93 ± 64.75), stage 2 (35.81 ± 29.45), and stage 3 (54.29 ± 64.41) with the control group (2.60 ± 0.84) was shown, while no significant difference was found between each stage.

Table 3 demonstrates non-significant differences ($p \geq 0.05$) in Na among the AKI patients of stage 1 (140.80 ± 3.61), stage 2 (138.24 ± 11.85), stage 3 (135.26 ± 11.14), and the control group (137.17 ± 3.61). Also, non-signifi-

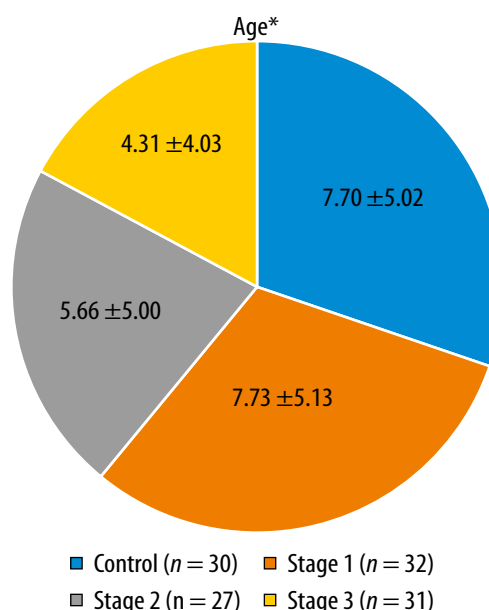


FIGURE 1. The age level in paediatric male patients with acute kidney injury stages compared to the control group

Least significant difference = 2.48
* Significant difference at $p \leq 0.01$

TABLE 1. The weight and height of paediatric male patients with acute kidney injury as compared to the control group using ANOVA

Parameters	Groups				p-value	LSD
	Control n = 30	Stage 1 n = 32	Stage 2 n = 27	Stage 3 n = 31		
Weight [kg]	27.85 ± 16.80	25.33 ± 15.30	18.65 ± 12.65	15.33 ± 10.10	0.001	6.96
Height [m]	1.22 ± 0.32	1.24 ± 0.35	1.06 ± 0.38	0.98 ± 0.29	0.01	0.17

LSD – least significant difference, N – number of sample

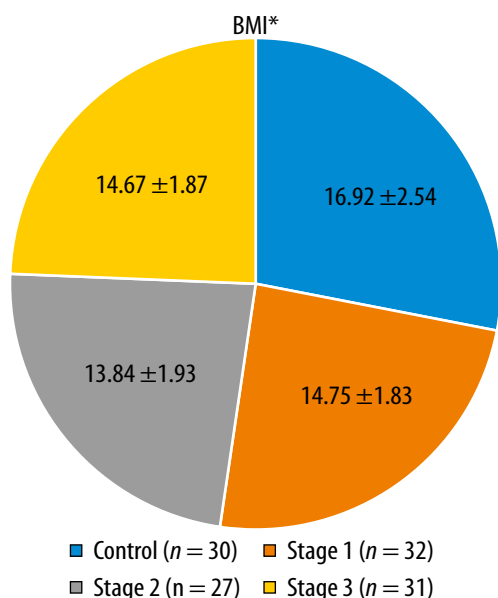


FIGURE 2. The level of body mass index in paediatric male patients with acute kidney injury stages compared to the control group

BMI – body mass index

Least significant difference = 1.03

* High significant difference at $p \leq 0.01$

cant differences ($p > 0.01$) among all stages with control and between each stage were shown.

A highly significant difference ($p \leq 0.001$) was found in K between stage 2 (4.96 ± 0.99) and stage 3 (5.16 ± 1.61) with control (4.25 ± 0.45) and between stage 1 (4.47 ± 0.92) and stage 3, while no significant difference between stage 1 with controls and between stage 1 and stage 2 and between stage 2 and stage 3.

A highly significant difference ($p \geq 0.05$) in Cl was found between stage 1 (111.11 ± 9.80) and controls (103.26 ± 2.81), while nonsignificant difference between stage 2 (107.31 ± 13.65) or stage 3 (106.32 ± 14.41) with controls and between stage 2 and stage 3 and between each stage.

The results shown in Table 4 demonstrate a highly significant difference ($p \leq 0.001$) in KIM-1 among stage 1 (1501.64 ± 417.02), stage 2 (1638.91 ± 354.85), and stage 3 (1474.16 ± 425.63), with the control group (716.64 ± 50.72), while no significant difference was seen between each stage.

Also, a highly significant difference ($p \leq 0.001$) was shown in cystatin C among AKI patients of stage 1 (12.52 ± 3.02), stage 2 (13.23 ± 2.42), and stage 3 (11.85 ± 3.04)

TABLE 2. The level of different renal function tests in paediatric male patients with acute kidney injury stages as compared to the control group using ANOVA

Parameters	Groups	N	Mean	SD	95% CI for mean		Min	Max	p-value	LSD
					Lower	Upper				
Creatinine [mg/dl]	Control	30	0.46	0.12	0.42	0.51	0.27	0.71	0.001	0.63
	Stage 1	32	0.63	0.18	0.56	0.69	0.34	0.90		
	Stage 2	27	0.97	0.36	0.83	1.11	0.50	1.80		
	Stage 3	31	3.18	2.36	2.32	4.05	1.00	9.69		
Urea [mg/dl]	Control	30	20.61	5.13	18.69	22.52	11.00	28.00	0.001	17.47
	Stage 1	32	61.10	17.99	54.61	67.59	43.00	112.00		
	Stage 2	27	74.85	24.42	65.19	84.51	41.00	123.80		
	Stage 3	31	174.79	62.30	151.94	197.64	82.40	325.10		
Albumin [g/dl]	Control	30	4.25	0.56	4.04	4.46	3.50	5.20	0.001	0.43
	Stage 1	32	2.64	0.98	2.29	3.00	1.01	4.24		
	Stage 2	27	2.57	0.89	2.22	2.92	1.00	4.80		
	Stage 3	31	2.91	0.91	2.58	3.25	1.40	4.70		
BUN [mg/dl]	Control	30	3.21	0.80	2.91	3.50	1.71	4.36	0.001	2.84
	Stage 1	32	9.49	2.78	8.49	10.50	6.69	17.42		
	Stage 2	27	11.64	3.80	10.14	13.15	6.38	19.26		
	Stage 3	31	27.19	9.69	23.63	30.74	12.82	50.57		
Urine output [ml/kg/hour]	Control	30	1.56	0.46	1.38	1.73	1.07	2.78	0.001	0.12
	Stage 1	32	0.39	0.06	0.37	0.42	0.28	0.49		
	Stage 2	27	0.38	0.05	0.36	0.40	0.30	0.49		
	Stage 3	31	0.14	0.09	0.11	0.18	0.01	0.31		
GFR [ml/min/1.73 m ²]	Control	30	109.71	13.06	104.83	114.59	91.32	147.50	0.001	4.67
	Stage 1	32	81.40	4.08	79.93	82.87	72.04	88.95		
	Stage 2	27	48.25	8.10	45.05	51.45	35.44	61.95		
	Stage 3	31	17.94	8.80	14.72	21.17	4.09	33.36		
CRP [mg/l]	Control	30	2.60	0.84	2.28	2.91	1.18	4.03	0.001	25.41
	Stage 1	32	33.93	64.75	10.59	57.28	1.70	345.00		
	Stage 2	27	35.81	29.45	24.16	47.46	6.63	118.30		
	Stage 3	31	54.29	64.41	30.66	77.91	4.56	350.00		

BUN – blood urea nitrogen, CRP – C-reactive protein, GFR – glomerular filtration rate, LSD – least significant difference, N – number of samples, SD – standard deviation

TABLE 3. The level of sodium, potassium, and chloride in paediatric male patients with acute kidney injury stages compared to the control group using ANOVA

Parameter	Groups	N	Mean	SD	95% CI for mean		Min	Max	p-value	LSD
					Lower	Upper				
Na [mmol/l]	Control	30	137.17	3.61	135.82	138.52	130.00	143.00	0.12	N.S
	Stage 1	32	140.80	8.32	137.80	143.80	122.00	170.00		
	Stage 2	27	138.24	11.85	133.56	142.93	114.00	165.00		
	Stage 3	31	135.20	11.16	131.10	139.29	108.00	163.00		
K [mmol/l]	Control	30	4.25	0.45	4.09	4.42	3.50	5.00	0.001	0.54
	Stage 1	32	4.47	0.92	4.13	4.80	2.39	6.10		
	Stage 2	27	4.96	0.99	4.57	5.36	3.10	6.98		
	Stage 3	31	5.16	1.61	4.57	5.75	2.27	9.10		
Cl [mmol/l]	Control	30	103.26	2.81	102.22	104.31	97.10	108.60	0.05	5.77
	Stage 1	32	111.11	9.80	107.58	114.64	92.00	140.00		
	Stage 2	27	107.31	13.65	101.91	112.72	79.00	135.20		
	Stage 3	31	106.32	14.41	101.03	111.60	71.00	136.00		

Cl – chloride, K – potassium, LSD – least significant differences, N – number of samples, Na – sodium, SD – standard deviation

TABLE 4. The level of kidney injury molecule-1 and cystatin C as predict biomarkers in paediatric male patients with acute kidney injury stages compared to the control group using ANOVA

Parameters	Groups	N	Mean	SD	95% CI for mean		Min	Max	p-value	LSD
					Lower	Upper				
Cystatin C [ng/ml]	Control	30	5.32	1.71	4.68	5.96	2.25	8.92	0.001	1.36
	Stage 1	32	12.52	3.02	11.43	13.61	4.55	18.01		
	Stage 2	27	13.23	2.42	12.28	14.19	4.54	17.54		
	Stage 3	31	11.85	3.04	10.73	12.97	4.52	17.19		
KIM-1 [pg/ml]	Control	30	716.64	50.72	698.49	734.79	611.87	812.933	0.001	181.75
	Stage 1	32	1501.64	417.02	1357.15	1646.13	430.97	2146.67		
	Stage 2	27	1638.91	354.85	1505.07	1772.76	471.72	2706.59		
	Stage 3	31	1474.16	425.63	1324.33	1623.99	468.09	2059.67		

KIM-1 – kidney injury molecule-1, LSD – least significant differences, N – number of samples, SD – standard deviation

with the control group (5.32 ± 1.71) and between stage 2 and stage 3, while no significant difference was seen between stage 1 and stage 2 and between stage 1 and stage 3.

Table 5 shows nonsignificant differences in stages 1, 2, and 3 between KIM-1 and age groups (1–4), (5–9), and (10 and more).

Table 6 shows nonsignificant differences in stages 1, 2, 3 between cystatin C and age groups (1–4), (5–9), and (10 and more).

Table 7 shows highly significant differences ($p \leq 0.001$) in age among all age groups (1–4), (5–9), and (10 and more), (1.85 ± 0.88), (6.80 ± 1.62), and (13.42 ± 1.98), respectively, in stage 1. Also, stages 2 and 3 showed highly significant differences ($p \leq 0.001$) in age among all age groups.

Table 8 shows highly significant differences ($p \leq 0.001$) in weight among all age groups (1–4), (5–9), and (10 and

more), (9.15 ± 2.25), (20.50 ± 5.50), and (42.83 ± 6.12), respectively, in stage 1. Also, stages 2 and 3 found highly significant differences ($p \leq 0.001$) in the weight among all age groups.

Table 9 shows highly significant differences ($p \leq 0.001$) in height among all age groups (1–4), (5–9), and (10 and more), (0.81 ± 0.08), (1.22 ± 0.16), and (1.61 ± 0.08), respectively, in stage 1. Also, stages 2 and 3 showed highly significant differences ($p \leq 0.001$) in weight among all age groups.

Table 10 shows highly significant differences ($p \leq 0.001$) in the BMI among all age groups (1–4), (5–9), and (10 and more), (13.88 ± 1.34), (13.61 ± 0.85), and (16.43 ± 1.58), respectively, in stage 1, while there is a non-significant difference in BMI among age groups in stages 2 and 3.

Table 11 shows the GFR by sCr among AKI patients in each stage of AKI to be (81.40 ± 4.08), (48.25 ± 8.10), and

TABLE 5. Comparison of kidney injury molecule-1 for acute kidney injury stages according to age group classification using ANOVA

Parameters	Stage	Ages	N	Mean	SD	p-value
KIM-1 [pg/ml]	Stage 1	1–4	10	1645.15	450.97	N.S
		5–9	10	1405.66	582.06	
		10 and more	12	1462.04	133.00	
	Stage 2	1–4	15	1683.88	462.36	N.S
		5–9	4	1610.65	179.58	
		10 and more	8	1568.71	126.11	
	Stage 3	1–4	20	1405.72	517.88	N.S
		5–9	7	1590.32	72.49	
		10 and more	4	1613.08	118.69	

KIM-1 – kidney injury molecule-1, LSD – least significant differences, N – number of sample, NS – non-significant, SD – standard deviation

TABLE 6. Compare the cystatin C for acute kidney injury stages according to age group classification using ANOVA

Parameters	Stage	Ages	N	Mean	SD	p-value
Cystatin C [mg/l]	Stage 1	1–4	10	1.85	0.88	N.S
		5–9	10	6.80	1.62	
		10 and more	12	13.42	1.98	
	Stage 2	1–4	15	12.57	2.50	N.S
		5–9	4	14.14	2.28	
		10 and more	8	14.03	2.21	
	Stage 3	1–4	20	11.46	3.53	NS
		5–9	7	11.82	0.78	
		10 and more	4	13.86	2.44	

LSD – least significant differences, N – number of samples, NS – non-significant, SD – standard deviation

TABLE 7. Classification of the stages of acute kidney injury paediatric male patients according to age group

Parameters	Stage	Ages	N	Mean	SD	p-value	LSD
Age (year)	Stage 1	1–4	10	1.85	0.88	0.001	1.40
		5–9	10	6.80	1.62		
		10 and more	12	13.42	1.98		
	Stage 2	1–4	15	1.84	1.04	0.001	1.85
		5–9	4	6.55	1.86		
		10 and more	8	12.38	2.45		
	Stage 3	1–4	20	1.78	1.01	0.001	1.22
		5–9	7	7.00	1.41		
		10 and more	4	12.25	2.87		

LSD – least significant differences, N – number of samples, SD – standard deviation

(17.94 ± 8.80) in stage 1, stage 2, and stage 3, respectively compared in control groups (109.71 ± 13.06) demonstrated highly significant difference ($p \leq 0.001$) between all stages with control and between stages 2 and 3 with stage 1 and between stage 2 and stage 3.

The glomerular filtration rate by sCr and Cys C among AKI patients in each stage of AKI was (28.56 ± 4.71), (20.74 ± 2.99), and (12.48 ± 4.07) in stage 1, stage

2, and stage 3, respectively, compared in control groups (50.30 ± 7.73) demonstrated highly significant difference ($p \leq 0.001$) among all stages with control and between stage 2 and stage 3 with stage 1 and between stage 2 and stage 3, while a nonsignificant difference was seen between stage 1 and stage 2.

The glomerular filtration rate by Cys C among AKI patients in each stage of AKI was (7.41 ± 2.84), (6.70 ± 2.16),

TABLE 8. Compare the weight of paediatric male patients of acute kidney injury stages according to age group in different stages

Parameters	Stage	Age	N	Mean	SD	p-value	LSD
Weight [kg]	Stage 1	1–4	10	9.15	2.25	0.001	4.40
		5–9	10	20.50	5.50		
		10 and more	12	42.83	6.12		
	Stage 2	1–4	15	9.77	2.85	0.001	2.01
		5–9	4	18.00	5.35		
		10 and more	8	35.63	8.19		
	Stage 3	1–4	20	9.47	2.64	0.001	5.01
		5–9	7	20.86	6.18		
		10 and more	4	35.00	9.13		

LSD – least significant differences, N – number of samples, SD – standard deviation

TABLE 9. Compare the height of paediatric male patients of acute kidney injury stages according to age group in different stages

Parameters	Stage	Age	N	Mean	SD	p-value	LSD
Height [m]	Stage 1	1–4	10	0.81	0.08	0.001	0.10
		5–9	10	1.22	0.16		
		10 and more	12	1.61	0.08		
	Stage 2	1–4	15	0.78	0.22	0.001	6.34
		5–9	4	1.20	0.10		
		10 and more	8	1.53	0.09		
	Stage 3	1–4	20	0.80	0.12	0.001	0.13
		5–9	7	1.19	0.13		
		10 and more	4	1.53	0.10		

LSD – least significant differences, N – number of samples, SD – standard deviation

TABLE 10. Comparison of body mass index for acute kidney injury stages according to age group classification

Parameters	Stage	Age	N	Mean	SD	p-value	LSD
BMI [kg/m ²]	Stage 1	1–4	10	13.88	1.34	0.001	1.15
		5–9	10	13.61	0.85		
		10 and more	12	16.43	1.58		
	Stage 2	1–4	15	13.73	1.56	NS	
		5–9	4	12.25	1.42		
		10 and more	8	14.85	2.35		
	Stage 3	1–4	20	14.70	1.98	NS	
		5–9	7	14.50	1.72		
		10 and more	4	14.80	2.08		

BMI – body mass, index, LSD – least significant differences, N – number of samples, NS – non-significant, SD – standard deviation

TABLE 11. The glomerular filtration rate by serum creatinine of paediatric male patients with acute kidney injury patients as compared to the control group using ANOVA

Parameters	Group				p- value	LSD
	Control 1, n = 30	Stage 1, n = 32	Stage 2, n = 27	Stage 3, n = 31		
GFR by sCr [ml/min/1.73 m ²]	109.71 ±13.06	81.40 ±4.08	48.25 ±8.10	17.94 ±8.80	0.001	4.70
GFR by sCr and cystatin C [ml/min/1.73 m ²]	50.30 ±7.73	28.56 ±4.71	20.74 ±2.99	12.48 ±4.07	0.001	2.71
GFR by cystatin C [ml/min/1.73 m ²]	16.30 ±5.07	7.41 ±2.84	6.70 ±2.16	7.77 ±3.32	0.001	1.84

GFR – glomerular filtration rate, LSD – least significant differences, N – number of samples, sCr – serum creatinine, SD – standard deviation

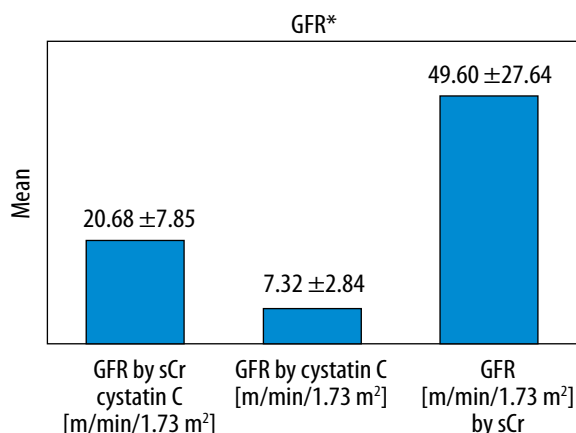


FIGURE 3. The level of glomerular filtration rate in paediatric male patients with acute kidney injury stages compared according to 3 equations

GFR – glomerular filtration rate, sCr – serum creatinine

Least significant difference = 4.87

* High significant difference at $p \geq 0.01$

and (7.77 ± 3.32) in stage 1, stage 2, and stage 3, respectively, compared in control groups (16.30 ± 5.07) demonstrated highly significant difference ($p \leq 0.001$) between all stages with control, while nonsignificant difference between each stage.

As shown in Figure 3, the result of GFR depends on the three-marker used detected as a decrease among AKI patients in each equation: GFR by sCr and cystatin C, GFR by cystatin C, and GFR (ml/min/1.73 m²) by sCr was (20.68 ± 7.85), (7.32 ± 2.84), and (49.60 ± 27.64), respectively. The analytical study demonstrated highly significant variations ($p \leq 0.001$) between patient groups.

Table 12 shows a positive correlation ($r = 0.366$) and ($r = 0.236$) between KIM-1 and urea and creatinine, respectively. A negative correlation ($r = -0.458$), ($r = -0.629$), ($r = -0.119$), ($r = -0.403$), and ($r = -0.503$) existed between KIM-1 and albumin, urine output, age, BMI, and GFR, respectively.

However, a positive correlation – ($r = 0.366$) and ($r = 0.214$) – existed between Cys C and urea and creatinine, respectively, and a negative correlation – ($r = -0.453$), ($r = 0.366$), ($r = -0.675$), ($r = -0.039$), ($r = -0.452$), and ($r = -0.522$) – existed between Cys C and albumin, BUN, urine output, age, BMI, and GFR, respectively.

DISCUSSION

In the current study, nutritional status measurements of growth parameters of enrolled children showed nonsignificant differences between the patient and control groups in age and height, but there was significant variation in weight and BMI between patient and control groups that related the majority of paediatrics with AKI suffer malnutrition and inactivity with a nutritional deficiency in comparison with paediatrics with normal or moderate dietary deficiency and this agreement with

[25], BMI was the most effective factor in predicting varying degrees of malnutrition agreement with [26] and disagreement with [27] demonstrated that high BMI increases the risk of AKI.

This study found that KIM-1 has increased concentrations in AKI patients related to KIM-1 is expressed in low levels in the kidney and other organs in normal kidneys. Nonetheless, its expression is accentuated in kidney injury because it is an enzyme released by tubular cell damage [1]. Kidney injury molecule-1 is released into the circulation following kidney proximal tubule damage. Tubular cell polarity is lost after damage, and KIM-1 may be discharged directly into the interstitial; furthermore, increased trans-epithelial permeability after tubular injury causes tubular contents to seep back into the circulation. In addition, increased microvascular permeability contributes significantly to the pathogenesis of kidney damage. In renal microvascular endothelial cells, the actin cytoskeleton architecture is disrupted, with loss of cell-cell and cell-matrix adhesion junctions, and endothelial cells are separated from the basement membrane, allowing KIM-1 to enter the circulation. The current study found that elevated levels of KIM-1 can be detected in the blood and can be used as a biomarker of kidney injury [28]. Kidney injury molecule-1 is secreted by inflammatory cells as macrophages that enter the kidneys during the inflammatory phase and are markedly up-regulated in the proximal tubule in the post-ischaemic kidney. After all, this pro-inflammatory mediator is produced because of tissue damage, which serves as a biomarker for early detection of kidney injury [14]. After the reduction in renal blood flow, the epithelial cells are unable to maintain sufficient intracellular adenosine triphosphate (ATP) levels for essential metabolic processes. This ATP depletion leads to cellular damage and, if severe enough, can cause cellular death through necrosis or apoptosis. During an ischaemic event, the proximal tubular cells are the most commonly damaged, although all segments of the nephrons are potentially affected [10]. There are numerous grounds to believe that KIM-1 is released into the circulation following kidney proximal tubule damage. Tubular cell polarity is lost after damage, and KIM-1 may be discharged directly into the interstitial. Furthermore, increased trans-epithelial permeability after tubular injury causes tubular contents to seep back into the circulation. In addition, increased microvascular permeability contributes significantly to the pathogenesis of kidney damage and this agrees with [28] but disagrees with [29], which demonstrated a decrease in KIM-1 in kidney injury in their study.

This study proved that cystatin C was found to increase concentrations AKI patients in compared to normal. That is because cystatin C is produced at a constant rate in all nucleated cells investigated, freely filtered by the glomeruli, and almost completely reabsorbed in the proximal tubule. In normal kidneys, it is expressed in

TABLE 12. Correlation between kidney injury molecule-1 and cystatin C with parameters using Spearman correlation

Parameter	Age	BMI	sCr	Urea	Albumin	BUN	Urine output	GFR by sCr	Na	K	Cl	CRP	GFR by sCr and Cys C	KIM-1
BMI	0.401**													
Creatinine	-0.025	-0.179												
Urea	-0.218*	-0.234*	0.754**											
Albumin	0.167	0.297**	-0.109	-0.336**										
BUN	-0.218*	-0.234*	0.754**	1.000**	-0.336**									
Urine output	0.035	0.337**	-0.423**	-0.622**	0.496**	-0.622**								
GFR by sCr	0.298**	0.373**	-0.660**	-0.811**	0.392**	-0.811**	0.750**							
Na	0.056	-0.098	-0.259**	-0.145	0.057	-0.145	-0.019	0.141						
K	-0.178	-0.083	-0.029	0.208*	-0.045	0.208*	-0.240**	-0.292**	0.124					
Cl	-0.161	-0.210*	-0.223*	-0.026	-0.026	-0.026	-0.136	-0.026	0.835**	0.172				
CRP	0.007	-0.164	0.243**	0.412**	-0.108	0.412**	-0.323**	-0.325**	-0.063	0.033	0.003			
GFR by sCr and cystatin C	0.305**	0.472**	-0.555**	-0.748**	0.497**	-0.748**	0.818**	0.918**	0.068	-0.261**	-0.096	-0.347**		
KIM-1	-0.119	-0.403**	0.236**	0.366**	-0.458**	0.366**	-0.629**	-0.503**	-0.065	0.138	0.103	0.279**	-0.690**	
Cystatin C	-0.039	-0.452**	0.214*	0.366**	-0.453**	0.366**	-0.675**	-0.522**	-0.002	0.160	0.111	0.278**	-0.761**	0.896**

BUN – blood urea nitrogen, Cl – chloride, CRP – C-reactive protein, GFR – glomerular filtration rate, K – potassium, KIM-1 – kidney injury molecule-1, Na – sodium, sCr – serum creatinine

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

low levels in the kidney and other organs, but its expression is elevated in kidney injury related to decreased GFR, because that Cystatin C can be eliminated almost exclusively from the bloodstream by glomerular filtration in the kidney, if kidney function and GFR decline, the blood levels of cystatin C increase. Kidney dysfunction increases the risk of cardiovascular disease and death, and this is in agreement with [10–14]. Cys C is believed to be neither actively secreted into the tubular lumen nor reabsorbed into the plasma. After filtration, Cys C is normally completely reabsorbed by proximal renal tubular epithelial cells, through megalin receptor-induced endocytosis, and catabolised. There is virtually no detection of CysC in the urine; however, it can be measured. Indeed, elevated urine Cys C may indicate tubular epithelial damage, and it has been proposed as an additional urine biomarker for AKI [30]. Cystatin C is an endogenous biomarker of renal function produced by all nucleated cells at a near-constant rate, independent of muscle mass, and it is cleared from circulation through glomerular filtration without reabsorption or secretion. CysC performs equally as sCr as a marker of renal function in most AKI cases and outperforms sCr in some cases [31], and this study is in disagreement with [29], which demonstrated a decrease of cystatin C in kidney injury.

This study demonstrates that developed serum creatinine in AKI patients is defined as an increase in serum creatinine, and this is because sCr is the product of creatine metabolism with a small molecular weight, most of which passes through glomerular filtration, and almost all the sCr formed in the body can be excreted by urine. Reduced cardiac output and decreased blood volume due to diuretic use result in renal insufficiency, decreased GFR, and increased sCr [24]. Creatinine elimination by the kidney and the kidney damage causes an accumulation of serum creatinine. Nonetheless, the diagnosis and stratification of AKI are made by obtaining serum creatinine levels, and this agrees with [10] but disagrees with [29], in which no changes in sCr were observed in their study of kidney injury.

An increase in blood urea related to urea is its product of proteins and nitrogen metabolism, urea is the most abundant substance in the blood of uremic people [32], and due to the decline of GFR as a result of early cellular damage, which also lowers total renal function. This causes a decrease in urine output as well as elevated serum urea levels [33]. This phase begins with the initial insult or injury to the kidneys. It is characterised by a decrease in renal blood flow and/or direct damage to the kidney tissue. Kidney function may decline, but it is still potentially reversible with appropriate interventions [34].

A study demonstrated an increase of BUN related to its filtration by GFR and reabsorption by renal tubular filtration, A serum urea level can be represented as a molar concentration or even as a mass concentration the levels of serum mass concentration can be defined for the en-

tire urea molecule or nitrogen equivalents (BUN) = 60/28 ratio ($O=C(NH_2)=urea$ that molecular weight = 60 and there are 2 molecules of nitrogen – N, = 28 gram) [32], BUN can change quickly enough during injury because individuals with normal renal function have a functional reserve that compensates for nephron injury [8], and this agrees with [35]. The normal cause of increased urea nitrogen is ingestion of food with a high nitrogen content, GFR, hypovolaemic shock, heart failure, gastrointestinal haemorrhage, fever, or increased catabolism.

To diagnose and stratify AKI, serum creatinine levels and urine output are assessed. A decrease in urine output alone is insufficient for AKI diagnosis, because its sensitivity and specificity are too low to confirm a diagnosis. It is crucial to consider that a healthy renal functional reserve can mitigate the increase in serum creatinine. This is supported by [29], which showed that AKI is characterised by an elevation in sCr or changes in urine output.

The study used 3 formulas to estimate GFR and demonstrated a highly significant reduction in GFR in AKI patient groups compared to the control groups in each of the formulas related to pre-renal, renal, and post-renal AKI causes are summarized by the pathophysiology of AKI, which varies based on the various factors linked to its development a network of capillaries that perform glomerular filtration – the process of filtering waste products and other molecules like proteins. The removal of bodily wastes is the foundation of glomerular filtration [10].

Renal blood flow is primarily regulated by multiple factors that involve extrarenal processes such as vascular tone, neurohormonal processes, and vasodilator/vasoconstrictor substances, among others. Hence, failure of any of these mechanisms will lead to hypoxia and then decreased blood flow, causing a decrease in glomerular filtration to produce the normal quantity of urine AKI is not a single disease, but instead consists of a loose syndrome – a collection of conditions including sepsis, cardiovascular causes, nephrotoxicity, urinary tract obstruction, and in short – anything that can cause GFR to be reduced quickly this agreement with [36]. Three markers to estimate GFR in patient groups and control groups and compare these three formulas. The first is performed by using sCr, which is filtered by the glomerular and almost completely reabsorbed in the proximal tubule, and the second by using cystatin C – another element that is filtered by the glomerular and in the proximal tubule completely reabsorbed, and that constant production and renal elimination make it an excellent biomarker of glomerular filtration, and this agrees with [10]. The third formula by using sCr and cystatin C formula [37].

This result is in agreement with [38], in which a slight increase in Na electrolyte was found in AKI patients, related to GFR changes, the excessive supply of Na to the renal tubules disrupts the balance between oxygen nutrient production and demand, resulting in damage

to tubular epithelial cells and leading to oxidative stress [39, 40], it disagrees with [40], which demonstrated that electrolyte disorders are associated with worse outcomes, with increased hospitalisation length and mortality, and [41] demonstrated that hypernatraemia is a frequent condition of life-threatening potential, found to occur in 9% of ICU patients. Hypernatraemia can cause peripheral insulin resistance, hepatic gluconeogenesis impairment, neuropsychiatric impairment, and cardiac contractility dysfunction, while hyponatraemia occurs due to a combination of inappropriate secretion of antidiuretic hormone and gastrointestinal fluid loss from vomiting and diarrhoea [40].

This study shows the increase of K related to many complications that can result from AKI, including metabolic acidosis, and elevated blood K levels with decreased excretion [1], and disagrees with [40] show hypokalaemia and demonstrated that electrolyte disorders are associated with worse outcomes, with increased hospitalisation length and mortality. Hyperkalaemia is a well-known complication in AKI, and it should be recognised instantly to prevent respective patients from severe cardiac arrhythmias. The data on AKI risk and outcomes of initial hypokalaemia are quite limited, in contrast [42]. Hyperkalaemia and K variability are probably AKI predictive. It must be prepended that serum K levels change in response to the intake of diuretics and/or angiotensin-converting enzyme/angiotensin II type 2 inhibitors. This aspect needs to be considered if electrolyte disturbances are diagnosed before AKI. A distinct electrolyte disorder may reflect drug-induced effects (e.g. K and volume depletion), ultimately responsible for AKI. The same applies to AKI-associated alterations in the renin-angiotensin axis, which also modifies electrolyte serum levels [42].

The study shows an increase of Cl related to the compromised ability to concentrate or dilute urine. In AKI, Cl levels may be elevated or decreased, depending on the underlying cause and severity of the injury. Elevated Cl levels may be associated with dehydration, while decreased levels may be due to fluid overload or impaired renal function, and this agrees with [43].

The study results show a decrease in albumin related to malnutrition in patients with AKI, which causes hypoalbuminaemia and is associated with the effect of other factors such as proteinuria – a common problem in patients with renal failure. Several studies found that was associated with higher urine albumin concentrations and a decrease in serum, an early indicator of renal damage [28]. The specificity of albumin level as a nutritional marker decreases in cases of inflammation and fluid overload, and acidaemia also affects serum albumin levels, which agrees with [26, 27].

The study demonstrated an increase in CRP related to AKI as the spread of inflammatory processes from the kidney to other organ systems also further increases the white blood cells count. This may be due to up-

regulation and the presence of interleukin-6 (IL-6) and cytokines such as tumour necrosis factor- α (TNF- α) in blood, which participate in inflammation in the uremic state [9]. AKI as the spread of inflammatory processes from the kidney to other organ systems is also affected by metabolic changes and undernutrition. Therefore, undernourished patients are more vulnerable to AKI, and this is in agreement with [27], which identified that nutritional markers (a low energetic intake, higher C-reactive protein level, etc.) are significantly associated with risk of death in AKI patients. Studies have demonstrated that malnutrition is related to poor prognosis in AKI, and CRP has a direct and significant relationship with malnutrition [26–44]. However, the elevation of traditional markers of inflammation such as CRP level is also associated with an increased risk of heart failure and mortality after acute myocardial infarction – one of the prerenal causes of AKI [45].

The study also demonstrates a correlation between KIM-1 and the biomarkers of the kidney. The positive correlation between KIM-1 and urea, creatinine, and BUN related to KIM-1 is an inflammatory biomarker, and elevated levels of KIM-1 can be detected in the blood and can be used as a biomarker of kidney injury [46], and each of urea according to [17], creatinine according to [10], and BUN elevated related to that excreted from the body depended on kidney filtration and due to kidney defect increase in blood. In contrast, the negative correlation between KIM-1, albumin, and urine output related to the increase of KIM-1 in AKI condition related to the presence of inflammation, and reduction in albumin was associated with inflammatory cytokine activation, which promotes protein degradation. Blood albumin levels can drop as a result of proteinuria, which is exacerbated by elevated blood pressure, while the decrease of urine output related to oliguria is the production of inadequate volumes of urine in AKI patients agreement with [26], a negative correlation between KIM-1, BMI, and GFR related to the increase of KIM-1 in kidney injury compared to a decrease each of BMI and GFR related to malnutrition presence in kidney injury patients that cause low weight and BMI [23], while a decrease in GFR one of the important markers of kidney injury [19], on the other hand, an increase of KIM-1 is released into the circulation following kidney proximal tubule damage [14].

Also, the study demonstrates the correlation between cystatin C and another biomarker of the kidney. The positive correlation between Cys C and creatinine, urea, and BUN is related to that each of them is a glomerular filtration biomarker cystatin C levels increase earlier than urea and creatinine when renal is injured [26], while the indirect correlation between albumin and urine output related to a decrease each of them in kidney injury, a negative correlation between Cys C, BMI, and GFR related to an increase in Cys C, which is considered an early marker for the diagnosis of AKI [47, 48], while a decrease in each

BMI due to malnutrition accompanied with kidney injury and decrease of GFR.

LIMITATIONS

In this study, it was a challenge to collect the AKI cases, particularly in the paediatric population, and it was difficult to obtain urine output with a catheter in younger male patients. The study was carried out in a single outpatient centre with a small sample size. As a result, the outcome of the study might not include all patients with acute kidney injury.

RECOMMENDATIONS

A future study is to be done on a larger sample size to give more accurate results; recommended to increase the age of patients and recommended to select females. Also recommended to study the AKI in infants after birth especially that may be related to pregnancy problems. For early treatment and to prevent complications, recommended to use serum cystatin C, which is considered the gold standard for GFR because it is more accurate and useful and not affected by gender, age, and other factors. Future studies to validate the sensitivity and specificity of KIM-1 and cystatin C as diagnostic biomarkers and as associated with the severity of different kinds of clinical renal injury could improve knowledge on these matters. Recommended to use the same biomarkers in adult or paediatric female patients with AKI or CKD. Future studies on other novel biomarkers such as urinary dickkopf-3, and chemokine ligand 2 in adults or paediatric males and females with AKI, or CKD.

CONCLUSIONS

Increased concentrations of cystatin C and KIM-1 biomarkers in all stages of AKI patients, Early detection and intervention are considered crucial to decrease the damage caused and reduce the chance of complications and mortality. Serum cystatin C adds more value than other kidney function markers in the early stages of AKI patients because it is more accurate for the measurement of GFR than creatinine and is less affected by gender, age, muscle mass, and food. Significant positive correlation between all biomarkers with some renal tests (urea, creatinine, BUN, and CRP), while significant negative correlation with (albumin, GFR, and urine output). There is a loss of growth factors such as BMI in patients with AKI, and this is related to many symptoms associated with AKI loss of activity, and malnutrition.

Prompt diagnosis and treatment of the disease will slow down the progression of the disease and prevent it from causing lasting complications, such as chronic kidney failure. Therefore, we are looking for diagnostic methods for early AKI identification. It is increasingly

evident that repeated AKI can lead to the development of CKD and eventually end-stage renal disease through a progressive decline in renal function without apparent symptoms in the early stages. Acute kidney injury is treated with renal replacement therapy (RRT). Malnutrition is highly prevalent in patients with acute kidney injury, especially in those receiving RRT.

DISCLOSURES

1. This study protocol was accepted after it was reviewed by a medical ethics committee at the University of Karbala College of Applied Medicine. Also, the study obtained research ethics permission from Karbala Children's Teaching Hospital. The study objectives were described to all participants, and verbal approval was obtained from each of them. Sampling, processing, and laboratory biochemistry assay investigations for the studied parameters were carried out in the laboratories of the previously mentioned hospitals. They were informed about the process and any potential risks involved. The local ethics committee reviewed and approved the study protocol, subject data, and permission form. This was done by document number 292 dated 10/10/2023.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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