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Original Article

# Serum Cystatin C and N-Acetyl-Beta-(D)-Glucosaminidase as Bio-markers in the Diagnosis of Acute Kidney Disease in Children

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## Abstract

**Background:** Acute kidney injury (AKI) is one of the main public health issues all over the world. Its accurate prevalence in children and adolescents in India is unknown. The main cause of death in children with kidney failure is cardiovascular disease and infections. Unfortunately, AKI is asymptomatic, and their signs and symptoms become evident only when a great proportion of kidneys have lost their function. Hence, the early diagnosis and treatment of AKI in children are of paramount importance. Many physicians base their diagnosis is based on reduced urine output, and serum creatinine (Scr) levels apart from the clinical symptomatology. Measuring the serum level of certain biomarkers as a new method for early diagnosis of AKI, among which major attention has been drawn to Cystatin C and N-acetyl-beta-(D)-glucosaminidase (NAG).

**Aim of the Study:** The aim of the study was to evaluate the roles of Scr, Cystatin C, and NAG levels in the early diagnosis of AKI.

**Materials and Methods:** A total of 62 children with AKI and 50 healthy children as control group were included to study the importance of biomarkers in its diagnosis. In addition to, Scr used in the protocol for the diagnosis of AKI, Cystatin C and NAG estimations were done for all the children. The lab values were analyzed to find the specificity and sensitivity of the biomarkers in relation to Scr.

**Observations and Results:** Among the 112 children included in this study, Group A was 62 children with clinical features of AKI and 50 healthy children as control Group B. In Group A there were 38 (61.29%) male and 24 (38.70%) female children. The mean age was 08.65 ± 2.40 years in males and 08.03 ± 2.15 years in females. The mean body surface area was 0.59 ± 0.32. The mean body mass index was 16.93 ± 1.85. There was no statistical difference in the demographic features (*P* taken significant at <0.05). The most common clinical symptom was decreased urine output in 57/62 (91.93%), swelling legs, ankle, and feet in 48/62 (77.41%). The most common cause of AKI was post-renal 20/62 (32.25%), acute glomerulonephritis in 16/62 (25.80%), and nephrotoxic drugs in 13/62 (20.96%) children. The mean Scr was 03.23 ± 1.25 mg/dL in Group A and normal 0.6.10 ± 0.15 mg/dL in Group B children. The mean urine creatinine was 4.14 ± 1.50 g/L in Group A and 1.1 ± 0.65 g/L in Group B children. The mean Serum Cystatin C was 1.59 ± 0.64 mg/L in Group A and 0.63± 0.17 in Group B children. The mean values of NAG were 149.61 ± 22.84 in Group A and 19.0 ± 2.80 in Group B children. The U.NAG/U. creatinine ratio was 526.62 ± 78.49 in Group A and 53.12 ± 7.35 Group B children. The specificity of Scr was 78.94% and sensitivity was 86.36%. Cystatin C as a diagnostic marker was with a specificity of 60% and sensitivity of 96.49%. NAG as a diagnostic marker was with a specificity of 66.66% and sensitivity of 96.55%. U.NAG/U creatinine as a diagnostic marker was with a specificity of 71.42% and sensitivity of 94.54%.

**Conclusions:** Cystatin C and urinary NAG have an acceptable diagnostic value for early detection of AKI when compared to Scr in children. Since the serum level of Cystatin C and urinary NAG raises within the first 24 h of admission in patients with AKI, this biomarker can be a suitable alternative for traditional diagnostic measures.

**Key words:** Acute kidney disease, Cystatin C and N-acetyl-β-glucosaminidase, Glomerular, Nephrotoxic, Serum creatinine, Tubular

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## INTRODUCTION

Acute kidney injury (AKI) is defined as an abrupt failure of kidney function with increased serum creatinine (Scr) levels with or without reduction in urine output. The damage may be mild to severe requiring renal replacement therapy.

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The causes may be pre-renal, intrinsic renal or post-renal. A thorough clinical history, recent usage of nephrotoxic medications and existing systemic illnesses would help in the diagnosis. Physical examination should include assessment of intravascular volume status, and skin rashes indicating systemic illnesses. Initially, measurement of Scr, complete blood count, urinalysis, and fractional excretion of sodium may be helpful. Ultrasound examination of kidneys should be done to know the status of kidneys and post-renal obstruction.[1] Current diagnostic criteria include acute loss of renal function with an increase in Scr levels within 48 h after injury ≥0.3 mg/dl (≥26.5 μmol/L)) or increase in Scr to ≥1.5 times baseline within the previous 7 days or urine output <0.5 mL/kg/h for 6 h.[2,3] The various complex causes of AKI include renal medullary ischemia caused by insufficient renal perfusion and renal vasoconstriction, decreased glomerular filtration rate (GFR), renal tubular obstruction, and remodeling, and metabolic changes of renal tubular epithelial cells. The kidney ischemia/reperfusion (I/R) is regarded as an important process in the causation of AKI today. The factors resulting in reduced I/R ratio are (1) vascular factors: Renal vasoconstriction; decreased renal blood flow; drop in glomerular blood pressure resulting in lateral medullary ischemia, leading to aggravation and activation of tubuloglomerular feedback; ischemic tissue damage; or cell necrosis. (2) Renal tubule factors: Including obstruction of renal tubules, reabsorption dysfunction, and renal interstitial inflammation.[4] Similarly, oxidative stress response activated by inflammatory mediators produced from damaged epithelial cells and the release of various vasoconstrictor substances further aggravates the ischemic damage.[4] Traditionally, Scr is used as a biochemical marker for the diagnosis of AKI, but Scr is not sensitive enough for early monitoring. Elevated Scr level is the consequence of loss of glomerular ultrafiltration capacity. As observed above several AKI cases occur due to acute renal tubular necrosis caused by ischemia or toxic substances, but they do not directly correlate with glomerular damage. On the other hand, the glomerulus has powerful compensatory capacity, and Scr usually begins to rise days or even weeks after AKI onset when GFR decreases by one-third to half, but not obvious in the diagnosis time window (48 h).[5] In addition, Scr level can be influenced by several interference factors such as age, sex, race, pre-renal factors, muscle mass, metabolism, and nutrition status.[6] N-acetyl-β-glucosaminidase (NAG) is a proximal tubule lysosomal enzyme and has been extensively studied and has proven to be a sensitive, persistent, and robust indicator of tubular injury. Increased NAG levels have been reported with nephrotoxicant exposure[7] delayed renal allograft function, chronic glomerular disease, diabetic nephropathy,[8] as well as following cardiopulmonary bypass procedures.[9] Westhuyzen *et al*.[10] reported that urinary NAG levels (in addition to other tubular enzymes) were highly sensitive in detecting AKI in a population of critically ill adult patients, preceding increases in Scr by 12 h–4 days. The two advantages of using NAG are (*a*) sensitivity, subtle alterations in the epithelial cells in the brush border of the proximal tubules result in shedding of NAG into the urine, and the amount of shed enzyme can be directly correlated to tubular injury and (*b*) quantization, simple and reproducible enzymatic assays are well established to measure the analyze calorimetrically using a spectrophotometer.[11] Cystatin C (CysC) belongs to the cysteine proteins inhibitors family with a 122-amino acid structure of low molecular weight (13 kDa). It is synthesized and released into plasma by all nucleated cells at a constant rate, can be freely filtered by the glomerulus due to its small size and positive charge, and is completely reabsorbed and degraded but not secreted by renal tubules. Hence, serum CysC is an early biomarker of AKI that can reflect the early changes in renal function and the decline of GFR. Initially, it was believed to be less influenced by factors such as weight, age, and gender. However, subsequent studies revealed that CysC concentration was higher in patients with greater height, body weight, age, and muscle content.[12] The time period of CysC increase was slightly different due to the initial cause of AKI and patient's age. A prospective single-center study showed that compared with a non-AKI group, CysC levels in pediatric patients with AKI were significantly increased 2 h after cardiopulmonary bypass surgery, suggesting that it may be a good biological marker for early diagnosis of AKI.[13] Similarly, Krawczeski *et al*.[14] found that CysC is a sensitive and specific marker for children who developed AKI 12 h after cardiac surgery. Haase-Fielitz *et al*.[15] found that blood CysC had 71% sensitivity and 53% specificity for diagnosing AKI in adults within 6 h after cardiac surgery. Recent studies showed that within 12 h after pediatric cardiopulmonary bypass surgery, serum CysC level was significantly elevated in patients who developed AKI.[16] Using 99 mTc-DTPA clearances as an index of glomerular filtration function, Uzun *et al*.[17] found that the sensitivity of CysC for diagnosing renal insufficiency was 82.8%, while that of Scr was only 68.2%; thus, CysC is easier to detect. Therefore, as a marker of kidney function, CysC is more ideal than Scr.[18] Some factors including storage conditions, muscle mass, age, gender, diet, infections, inflammation, and tumor may not affect serum CysC levels, but CysC may be influenced by thyroid dysfunction, immunosuppressant use (e.g., Glucocorticoids), smoking, systemic inflammatory response, and elevated C-reactive protein[19] Its widespread application in clinical has been limited because of lack of a standardized test. In the present study, both NAG and CysC were used in pediatric age group patients with AKI to evaluate their role as diagnostic factors.

### Type of the Study

This was a cross-sectional prospective and comparative study.

#### *Institute of the study*

The study was conducted at the Departments of Biochemistry and Pediatrics, Kannur Medical College, Anjarakandy, Kannur, Kerala.

#### *Period of the study*

The study duration was from July 2015 to June 2017 (2 years).

## MATERIALS AND METHODS

A total of 62 children attending the department of pediatrics at a tertiary teaching hospital of Kerala with AKI were included in this study. A group of 50 healthy children attending the department of pediatrics for a regular health check-up were included as a control group. An institutional committee approval was obtained from the ethical committee before commencement to the study. An Ethical Committee approved consent form was used in this study.

### Inclusion Criteria

1. Children aged between 3 and 13 years were included
2. Children satisfying "RIFLE criteria" to diagnose AKI as quoted in reference[20] were included
3. Children with features of AKI occurring within 7 days were included.

### Exclusion Criteria

1. Children below 3 and above 15 years were excluded
2. Children with acute systemic illnesses were excluded
3. Children with diabetes mellitus, cardiac diseases, and metabolic disorders were excluded
4. Children with Chronic Kidney diseases were excluded
5. Children earlier treated for AKI were excluded
6. Children with a treatment history of tuberculosis or fungal infections were excluded
7. Children with a history of acute abdominal emergencies were excluded.

Serum and urine samples of these children were analyzed for Scr, Cystatin C, and Urinary NAG levels. Clinical history taking to include demographic and biometric data was done. Clinical examination was done to identify the symptoms and signs of AKI. Ultrasound examination of the kidney was done in all the children. Serum Cystatin C was analyzed using turbidimetric immunoassay for the quantitative determination in human serum and is based on the principle of the agglutination reaction. The test specimen is mixed with Cystatin C latex reagent (R2) and activation buffer (R1) and allowed to react. Presence of Cystatin C in the test specimen results in the formation of insoluble complex producing turbidity which is measured at a wavelength of 630 nm in semi-automated analyzer. The extent of turbidity corresponds to the concentration of Cystatin C in the specimen. The system was designed to detect Cystatin C concentration ranging from 0.5 to 8.0 mg/L. The lowest limit of detection was 0.33 mg/L; the lowest measurable Cystatin C concentration that can be distinguished from zero. The reference values for Cystatin C were determined to be 0.5 mg/L–1.05 mg/L. GFR was calculated using the following formula:

$$\text{GFR mL / min / 1.73 m}^2 = \frac{79.901}{\text{Cystatin C mg / L}}$$

No interface was observed with hemoglobin 8 Gms/L, bilirubin 420mg/L, and triglycerides 12.5 mmol/mL.

The urinary NAG levels were assessed by the colorimetric method by centrifuging the urine samples at 12,000 rpm for 10 min and storing the supernatants at −80°C. Urinary NAG (10 μL) was quantified by immunoblots with non-reducing 4%–20% gradient polyacrylamide gels (Bio-Rad Laboratories, Hercules, California) and monoclonal (1:1000; Antibody Shop, BioPorto Diagnostics, Gentofte, Denmark). The immunoblotting procedure (runtime, approximately 10 h) was used in this study. The urinary NAG values were expressed as the urinary NAG/creatinine ratio (U/L). Scr, the reference standard, was measured in the Columbia University Medical Center Core Laboratory using the Jaffe reaction. All the data were analyzed using standard statistical methods.

### Statistical Analysis

All data analysis was done using Microsoft Excel and the Statistical Package for the Social Sciences (version 16.0) Windows software. Mean ± standard deviation was calculated. Results were analyzed statistically for significance by independent *t*-test and Chi-square test. Moreover, Pearson correlation "r" test (correlation coefficient test) was done to assess the relation of biochemical laboratory investigations with demographic parameters. Student's *t*-test used to calculate *P* values. At $P < 0.05$, results were considered significant.

### Observations and Results

Overall, 112 children were included in this study. 62 children with clinical features of AKI were grouped as A and 50 healthy children as a control study grouped as B. There were 38 (61.29%) male and 24 (38.70%) female children. The children were aged between 3 and 15 years with a mean age of 08.65 ± 2.40 years in males and 08.03 ± 2.15 years in females. The demographic details are shown in Table 1. The body surface area of the children varied

**Table 1: The demographic details of the study groups (A-62; B-50=n-112)**

| Observations | Group A - 62 | | Group B - 50 | | *P* value |
|---|---|---|---|---|---|
| | Male-38 | Female - 24 | Male - 27 | Female - 23 | |
| Age (years) | | | | | |
| 3–6 | 6 | 5 | 5 | 4 | 0.073 |
| 7–10 | 19 | 9 | 8 | 9 | |
| 11–13 | 13 | 10 | 14 | 10 | |
| Socioeconomic status | | | | | |
| Low | 14 | 11 | 10 | 11 | 0.082 |
| Middle | 16 | 8 | 13 | 9 | |
| High | 8 | 5 | 4 | 3 | |
| Weight category | | | | | |
| Under weight | 12 | 9 | 9 | 8 | 0.076 |
| Normal weight | 15 | 8 | 7 | 9 | |
| Over weight | 11 | 7 | 11 | 6 | |
| BMI (Kg.m²), mean | 17.30±1.17 | 16.85±1.38 | 16.98±2.20 | 16.60±2.65 | 0.068 |
| Body surface area (M²) | 0.59±0.32 | 0.56±0.41 | 0.60±0.30 | 0.60±0.12 | 0.074 |

BMI: Body mass index

**Table 2: The clinical presentation of the study groups n-112 (A-62; B-50)**

| Observations | Group A n=62 (%) |
|---|---|
| Symptoms | |
| Decreased urine output | 57 (91.93) |
| Swelling legs, ankle, and feet | 48 (77.41) |
| Breathlessness | 46 (74.19) |
| Nausea | 35 (56.45) |
| Fatigue | 31 (50) |
| Palpitations | 21 (33.87) |
| Seizures | 13 (20.96) |
| Pain in the chest | 11 (17.74) |

**Table 3: The causes of AKI in the study n-62 (Group A)**

| Causes of AKI | Male n=38 (%) | Female-24 n=24 (%) |
|---|---|---|
| **Post-renal causes - 20** | 11 (28.94) | 9 (37.50) |
| Acute glomerulonephritis - 16 | 9 (23.68) | 7 (29.16) |
| Nephrotoxic drugs - 13 | 8 (21.05) | 5 (20.83) |
| Blunt injury to abdomen - 9 | 6 (15.78) | 3 (12.50) |
| Infections - 6 | 4 (10.52) | 2 (08.33) |

AKI: Acute kidney injury

from 0.41 ± 1.05 to 1.3 ± 1.75 with a mean of 0.59 ± 0.32. The body mass index was varying from 24.65 ± 3.50 to 16.80 ± 4.80 with a mean value of 16.93 ± 1.85. There was no statistical difference in the demographic features of both groups; the p values were more than 0.05 (p taken significant at <0.05), [Table 1].

The most common symptom observed in this study was decreased urine output in 57 (91.93%), swelling legs, ankle and feet in 48 (77.41%) children, breathlessness in 46 (74.19%), nausea in 35 (56.45%), and fatigue in 31 (50%) of the children. Other minor symptoms were palpitations in 21/62 (33.87%), seizures in 13/62 (20.96%), and pain in the chest in 11/62 (17.74%). The clinical symptoms and signs in the study group children were tabulated in Table 2.

The various causes observed as the etiology of AKI in the study showed 20/62 (32.25%) children with post-renal causes, 16/62 (25.80%) children with acute glomerulonephritis, 13/62 children with use of nephrotoxic drugs (20.96%), 9 children with blunt injury to abdomen (14.51%), and 6 children (09.67%) with infections [Table 3].

Laboratory investigations undertaken in the study included were urine dipstick which was positive in 18/62 (29.03%) of Group A and negative in all of Group B children whereas in Group B 8/50 (16%) showed epithelial cells which were normal. Urine samples showed that red cell casts were observed in 15/62 (24.19%), dysmorphic red cells in 10/62 (16.12%), white cell casts in 5/62 (08.06%), leukocytes >3 cells/field in 7/62 (11.29%), granular casts in 14/62 (22.58%), and renal tubular cells in 11/62 (17.74%) children. Renal ultrasound examination showed positive renal vessel obstruction in 7/62 (11.29%) children and ascites, raised intra-abdominal pressure >20mmHg in 4/62 (06.45%) children. There was raised white blood cells (WBC) count all the children of Group A with a mean value of 16.45 $10^3$/µl, whereas the WBC count was normal in the control group. The mean erythrocyte sedimentation rate was 28 in Group A and 14 in Group B children. The mean Scr was 03.23 ± 1.25 mg/dL in Group A and normal 0.6.10 ± 0.15 mg/dL in Group B children. The mean urine creatinine was 4.14 ± 1.50 g/L in Group A and 1.1 ± 0.65 g/L in Group B children. The mean blood urea was 45.79 ± 2.70 mg/L in Group A and 6.3 ± 0.96 mg/dL in Group B children. The mean serum Cystatin C was 1.59 ± 0.64mg/L in Group A and 0.63 ± 0.17 in Group B children. The mean values of NAG were 149.61 ± 22.84 in Group A and 19.0 ± 2.80 in Group B children. The U.NAG/U. Creatinine ratio was 526.62 ±

78.49 in Group A and 53.12 ± 7.35 Group B children [Table 4].

The mean Scr, Cystatin C, and NAG values obtained from children with various causes resulting in AKI were tabulated in Table 5.

In this study, the specificity of Scr as a diagnostic marker was with a specificity of 78.94% and sensitivity was 86.36%. Cystatin C as a diagnostic marker was with a specificity of 60% and sensitivity was 96.49%. NAG 96 as a diagnostic marker was with a specificity of 66.66% and sensitivity was 96.55%. U.NAG/U creatinine as a diagnostic marker was with specificity of 71.42% and sensitivity was 94.54% [Table 6]. This confirms the finding that renal biomarkers such as serum Cystatin C, and NAG are elevated much before Scr levels start rising and do not suffer from the disadvantage of creatinine blind area. In this way, it helps in early detection of kidney injury.

**Table 4: Baseline laboratory and ultrasonography results of children in the study Groups (A-62; B-50=n-112)**

| Investigations - Mean values | Group A - 62 | Group B - 50 | *P* value |
|---|---|---|---|
| Urine dipstick positive | 18 | Negative | |
| Urine microscopy positive in children | | Epithelial cells+in 08 | |
| Red cell casts+ | 15 | Negative | |
| Dysmorphic red cells+ | 10 | Negative | |
| White cell casts+ | 5 | Negative | |
| Leukocytes>3 cells/field± | 7 | Negative | |
| Granular casts+ | 14 | Negative | |
| Renal tubular cells+ | 11 | Negative | |
| Renal ultrasound | | | |
| Renal Doppler study | 7 | | |
| Ascites, raised intra-abdominal pressure >20 mmHg | 4 | Normal | |
| WBC count - $10^3$/μmL, mean value | 16.45±3.10 | 6.20 | 0.075 |
| ESR: mm/H, mean value | 28 | 14 | 0.083 |
| Scr: mg/dL, mean value | 3.23±1.25 | 06.10±0.15 | 0.031 |
| Urine creatinine: g/L, mean value | 4.14±1.50 | 1.1±0.65 | 0.043 |
| Blood Urea: mg/dL, mean value | 45.79±2.70 | 6.3±0.96 | 0.039 |
| Cystatin C: mg/L, mean value | 1.59±0.64 | 0.63±0.17 | 0.044 |
| Urinary NAG: U/L, mean value | 149.61±22.84 | 19.50±2.80 | 0.027 |
| U.NAG/U. creatinine ratio: U/g, Mean value | 526.62±78.49 | 53.12±7.35 | 0.019 |

WBC: White blood cells, ESR: Erythrocyte sedimentation rate, Scr: Serum creatinine, NAG: N-acetyl-beta-(D)-glucosaminidase

**Table 5: The serial Lab values of Scr (Cr), CYSC, and NAG in different causes of AKI in the study (n-62 Group A)**

| Causes of AKI | Mean values on admission | | |
|---|---|---|---|
| | Scr | Cystatin C | NAG |
| Post-renal causes-20 | 3.18±1.78 | 1.21±0.11 | 148.37±19.46 |
| Acute glomerulonephritis - 16 | 4.10±1.15 | 1.74±0.41 | 153.90±21.55 |
| Nephrotoxic drugs - 13 | 2.87±1.02 | 1.62±0.12 | 167.80±30.19 |
| Blunt injury to abdomen - 9 | 2.90±1.20 | 1.80±0.7 | 137.65±18.75 |
| Infections - 6 | 3.10±1.10 | 1.58±1.90 | 140.36±24.25 |

Scr: Serum creatinine, CYSC: Cystatin C, NAG: N-acetyl-beta-(D)-glucosaminidase, AKI: Acute kidney injury

## DISCUSSION

In the present study, the roles of Scr, Cystatin C, and NAG levels in the early diagnosis of AKI were conducted. All over the world, Scr is used initially to predict, diagnose and treat AKI in both adults and children. Scr is actually used as a measure of GFR in clinical medicine. Unfortunately, Scr concentrations are not determined only by glomerular filtration.[21] Scr levels are affected by renal handling, metabolism and methods used in its assessment.[22] It is also affected by muscle mass, body weight, and size especially in growing children.[23,24] Cystatin C, a non-glycosylated low molecular weight protein ($M_r$ 13.359),[25] is a proteinase inhibitor involved in the intracellular catabolism of proteins.[26] Unlike creatinine, Cystatin C is produced in all nucleated cells at a constant rate, freely filtered in the renal glomeruli and almost completely reabsorbed and catabolized in the renal proximal tubular cells.[26,27] Recent studies have shown that Cystatin C can be used as an endogenous marker of GFR and is a promising marker in children.[28-30] In hemodialysis patients, GFR values calculated from serum Cys-C show less variability than those calculated from Scr.[31] Cys-C is a good marker of GFR for an early identification of fetuses and children with urinary tract malformations.[32,33] Bladder dysfunctions are frequently associated with occult spina bifida. In a prospective study, serum Cys-C and creatinine were used to estimate kidney function in subjects with nervous system malformation. In these children, the estimation of GFR from Cys-C was superior to that from creatinine.[34] Compared with creatinine, Cystatin C facilitates the recognition of abnormal renal function in children as its reference range is constant beyond the 1st year of life. The higher levels of Cystatin C in the 1st year of life probably reflect the low GFR of neonates and infants.[35] NAG is a hydrolytic enzyme with a molecular weight of 130,000–140,000 Daltons. It is normally not filtered at the glomerulus. NAG is a widely distributed lysosomal enzyme, located predominantly in the renal proximal tubules.[36] NAG is a lysosomal hydrolysis product that plays an important role in the catabolism of both glycoprotein's and glycosaminoglycans.[37-39] This mechanism is mainly occurred in the proximal tubules and

**Table 6: The specificity and sensitivity of tests of Scr, CYSC, Nag, and U.NAG/U. creatinine ratio in study group; n-62 Group A**

| AKI | True positive | False positive | True Negative | False negative | Specificity (%) | Sensitivity (%) |
|---|---|---|---|---|---|---|
| Scr | 19 | 08 | 30 | 03 | 78.94 | 86.36 |
| Serum cystatin C | 55 | 02 | 03 | 02 | 60 | 96.49 |
| NAG | 56 | 01 | 02 | 02 | 66.66 | 96.55 |
| U.NAG/U. creatinine ratio | 52 | 02 | 5 | 03 | 71.42 | 94.54 |

Scr: Serum creatinine, CYSC: Cystatin C, NAG: N-acetyl-beta-(D)-glucosaminidase, AKI: Acute kidney injury

therefore can be interpreted as an indicator of functional disturbance of tubules.[40] In addition to its use as a marker of AKI, it is mainly a bacteriostatic substance released from secondary granulocytes of neutrophils.[41] Another study demonstrated an increase in urine and serum as a response to ischemia from renal tubular cells in the early period following ischemic damage, independent of the glomerular filtration; it was also shown to be a sensitive marker.[42] In the present study, the mean Scr was 03.23 ± 1.25 mg/dL in Group A and normal 0.6.10 ± 0.15 mg/dL in Group B children. The mean urine creatinine was 4.14 ± 1.50 g/L in Group A and 1.1 ± 0.65 g/L in Group B children. The mean blood urea was 45.79 ± 2.70 mg/L in Group A and 6.3 ± 0.96 mg/dL in Group B children. The mean serum Cystatin C was 1.59 ± 0.64 mg/L in Group A and 0.63 ± 0.17 in Group B children. The mean values of NAG were 149.61 ± 22.84 in Group A and 19.0 ± 2.80 in Group B children. The U.NAG/U. creatinine ratio was 526.62 ± 78.49 in Group A and 53.12 ± 7.35 Group B children. The serum Cystatin C levels also predict the AKI 1–2 days before the Scr level[43] and an increased urinary Cystatin C level has predicted the need for dialysis earlier than Scr.[44] In a study by Hari *et al.* they observed that to compare performance of combined creatinine and Cystatin C based equation with equations based on either Cystatin C alone or creatinine alone found that Cystatin C based equation has a better performance in estimation GFR than creatinine-based equation in children with AKI.[45] Serum Cystatin C showed a high correlation with measured GFR in young and older patients with CKD than creatinine. Thus, Cystatin C is a good alternative marker to creatinine in CKD patients.[46] Serum Cystatin C may be influenced by factors other than renal function alone, including serum C-reactive protein,[47] smoking[48] the subjects with very low GFR,[49] thyroid function,[50,51] immunosuppressive therapy,[52] and occupational exposure to toxic agents such as lead, cadmium, and arsenic[52]. Thus, clinicians must be cautious when interpreting Cystatin C levels alone if the subjects encounter these factors. In this study, the specificity of Scr as a diagnostic marker was with specificity of 78.94% and sensitivity was 86.36%. Cystatin C as a diagnostic marker was with specificity of 60% and sensitivity was 96.49%. NAG 96 as a diagnostic marker was with specificity of 66.66% and sensitivity was 96.55%. U.NAG/U creatinine as a diagnostic marker was with specificity of 71.42% and sensitivity was 94.54% [Table 6]. This confirms the finding that renal biomarkers such as serum Cystatin C and NAG are elevated much before Scr levels start rising and do not suffer from the disadvantage of creatinine blind area. In this way, it helps in early detection of kidney injury.

## CONCLUSIONS

Cystatin C and urinary NAG have an acceptable diagnostic value for early detection of AKI when compared to Scr in children. Since the serum level of Cystatin C and urinary NAG raises within the first 24 h of admission in patients with AKI, this biomarker can be a suitable alternative for traditional diagnostic measures.

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