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Early Detection of Nephropathy in Iraqi Pediatric Patients with Sickle Cell Anemia Using Novel Physiological Parameters

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Abstract

This study goal to estimate the diagnostic and prognostic characteristics of nephropathy markers (Cystatin C, Neutrophil Gelatinase Associated Lipocalin (NGAL), Beta-2 Microglobulin, N-Acetyl Beta-D-Glucosaminidase (NAG), Nephrin, and Kidney Injury Molecule 1(KIM-1)) in steady state and vaso-occlusive crisis (VOC) of sickle cell anemia. The study was included 70 children diagnosed with sickle cell anemia with age (3-18) years and 60 healthy children with age (3-18) years. Sickle cell anemia was divided into 35 patients with steady state and 35 with VOC. Enzyme-linked Immunosorbent Assay (ELISA) was used to estimate Cystatin C, Neutrophil Gelatinase Associated Lipocalin (NGAL), Beta-2 Microglobulin, N-Acetyl Beta-D-Glucosaminidase (NAG), Nephrin, and Kidney Injury Molecule-1 (KIM-1) in patients and control. The results show that the levels of cystatin C, NGAL, KIM-1, NAG, β2-microglobulin, and Nephrin had been significant increase in steady state patients (1.4±0.12, 6.2±1.35, 3.79 ±0.21 , 129±5.3, 4.3±1.2, and 4.5±0.3) respectively and VOC group (2.3±0.41, 10.35 ±0.21, 5.3±0.82, 83±4.81, 7.2±1.6, and 6.8±0.8) respectively compared with control (0.78±0.03, 3.8±1.03, 1.8±0.08, 72±4.62, 3.14±0.7, and 2.12±0.24) respectively. Based on these findings, we identified the best marker for routine screening to detect renal involvement during sickle cell anemia. Cystatin C, NGAL, and KIM-1 all exhibited high diagnostic accuracy in distinguishing patients with sickle cell anemia from healthy controls.

Key words: Nephropathy, Sickle cell anemia, Physiological parameters.

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Introduction

Sickle cell anemia (SCA) is one of the most prevalent hereditary hemoglobinopathies [1]. The disease hallmarks are Hemoglobin S polymerization, hemolysis, and vaso-occlusive crisis (VOC), which subsequently results in endothelial dysfunction, oxidative stress, ischemia reperfusion injury, and immune activation [2]. The brain, kidneys, and bones are among the organs that suffer increasing deterioration as a result of SCA [3]. One of the most dangerous side effects of SCA is sickle cell nephropathy (SCN). It is typified by early-life hyper-filtration, which leads to hypo-sthenuria [4]. At childhood age, sickle cell disease patients have proximal tubular dysfunction, supra-normal glomerular filtration rate (GFR), and reduced potassium excretion or urine acidification [5]. Sickle cell anemia Patients must be considered at risk for chronic kidney disease (CKD). Research indicates that gross proteinuria and end-stage renal disease are observed in 15–30% of SCD patients [6].

Serum creatinine and urea levels are often used in clinical settings to identify renal damage. The fact that these markers are impacted by age, sex, muscle mass, dehydration, and medications is a disadvantage, but [7]. Furthermore, unless at least half of the renal function is lost, the creatinine level does not rise [8]. In order for treatment interventions to effectively stop the course of renal damage, novel biomarkers are required for the early identification of sickle cell nephropathy, before the damage to the kidney becomes permanent [9]. Most promising of these biomarkers include Cystatin C, urine kidney injury molecule-1 (KIM-1), N-acetyl-b-D-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), β2-microglobulin and nephrin [10]. Cystain C more reliable method than creatinine when assessing GFR in people with SCD that is freely filtered in glomeruli [11].

Urine kidney injury molecule-1is a sensitive and specific biomarker for detecting kidney injury, particularly in the early stages of acute kidney injury. KIM-1 rises even in preclinical nephropathy and is highly selective for proximal tubular damage [12]. While, neutrophil gelatinase–associated lipocalin (NGAL), a newly discovered adipokine, is a member of the lipocalin superfamily [13]. NGAL may be produced by kidney tubular cells in response to different types of injury. It is closely linked to albuminuria [14]. NGAL is a new, sensitive indicator of renal tubular damage in both acute and chronic nephropathy, according to Devarajan and Colleagues [15].

N-acetyl-β-D-glucosaminidase is a lysosomal enzyme released during tubular damage. Elevated in SCD patients before overt nephropathy [16]. Nephrin is a transmembrane protein that demonstrates the structural integrity of the podocyte's cytoskeleton. Its presence in the urine is a sign of glomerular-specific injury [17]. In diabetic individuals, elevated nephrin may be a more sensitive indicator of nephropathy than albuminuria or decreased eGFR [18]. In SCD-afflicted adults and children, urinary nephrin levels had a positive correlation with albuminuria [19]. We searched the literature but couldn't locate any study on the impact of sickle cell anemia on kidney function in the

Iraqi population utilizing renal damage molecules. Therefore, this study was aimed at evaluating the diagnostic and prognostic characteristics of nephropathy markers (Cystatin C, NGAL, Beta-2 Microglobulin, KIM-1, NAG, and nephrin) in steady state and vaso-occlusive crisis (VOC) of sickle cell anemia.

Materials and methods

Participants and Study Design

This is a comparative study conducted to assess the renal functions of sickle cell nephropathy. We enrolled children with sickle cell anemia at Ramadi Hospital between January and June 2025, diagnosed by using hemoglobin electrophoresis.

This study included 70 children (31 males and 39 females) diagnosed with sickle cell anemia with age (3-18) years and 60 healthy children (28 males and 32 females) with age (3-18) years.

Sickle cell anemia was divided into 35 patients with steady state. In the prior 3 weeks to enrollment, who did not have any VOC, blood transfusion or acute illness, 35 VOC patients, defined as an episode of intense pain, were admitted within 24 h. Healthy controls who were matched for age and ethnicity and who did not have anemia, kidney-related illnesses, a history of chronic drug use, or a history of drug use during the previous two months were recruited. A preoperative examination for minor surgery, such circumcision or hernia repair, or a normal control check-up led to their admission to our hospital.

Individuals were excluded: If they were on blood transfusion programs or had cancer, diabetes mellitus, angiotensin II receptor blockers, or angiotensin-converting enzyme inhibitors.

Ethical approval

The College of Medicine's/ University of Anbar (Research Ethics Committee gave the study protocol ethical clearance (Certificate No: 48 on January 23, 2025). Before being included in the study, each participant provided written informed consent in compliance with the Helsinki Declaration's ethical guidelines.

Clinical characteristics of participants

Age, sex, blood transfusion, consanguinity, length of disease, frequency of hospitalization, vaso-occlusive crises, history of infections, symptoms of urinary (hematuria and polyuria), status of spleen, medication therapy, and complications were all covered in the comprehensive history of each patient. Additionally, a physical examination was performed, which included measurements of body weight, standing height, and Body Mass Index (BMI).

Blood and urine samples collection and examination

Children's venous blood was extracted upon admission, and serum was collected in plane tube. The blood was then allowed to clot at 25°C for 30 minutes, then centrifuged at 1200 g for 10 minutes, and stored at -70°C for further biomarker testing.

For a urinalysis, a spot urine sample was taken using a pee bag or container for older children. A urinary tract infection test was performed on the urine samples. After centrifuging the urine samples for three minutes at 3000 RPM, the samples were

analyzed under a microscope after sugar and albumin were detected using dipsticks. Urine that had been centrifuged was put into four different Eppendorf tubes and kept at -70 °C until ELISA testing. The modified Schwartz formula for children was used to calculate the estimated glomerular filtration rate, or eGFR. Serum creatinine (mg/dL) × height (cm) × 0.413 = eGFR (mL/min/1.73 m^2). Normal kidney function was defined as eGFR >90 mL/min/1.73 m2, and reduced renal function was defined as eGFR <90 mL/min/1.73 m^2.

Laboratory tests included complete blood count (CBC), white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), and hematocrit (Hct). Following biochemical analyses, urine albumin, serum urea, and creatinine were measured photometrically using an Abbott ARCHITECT C16000 analyzer device (Abbott Park, IL), while hemoglobin electrophoresis [using Hyrys_Sebia]. Afterwards, the following variables were measured by ELISA Human Cystatin C (Cat. No: EHCST3)from Thermo Fisher Scientific company/USA, NGAL Human ELISA Kit (Cat. No: KIT036),from Thermo Fisher Scientific company/USA, Human Beta-2 Microglobulin ELISA (Cat. No: SE120150) from Sigma-Aldrich USA, Human NGAL (Neutrophil Gelatinase Associated Lipocalin) ELISA Kit (Cat. No: ELK1063) from ELK biotechnology company/China, Human urine NAGase(N-Acetyl Beta-D-Glucosaminidase) ELISA Kit (Cat. No: ELK1917) from ELK biotechnology company/China, Human NPHN (Nephrin) ELISA Kit (Cat. No: ELK2043) from ELK biotechnology company/China, and Human urine Kim1(Kidney Injury Molecule 1) ELISA Kit (Cat. No: ELK11098) from ELK biotechnology company/China. The amounts of study markers in the samples were ascertained by comparing the samples' optical density (OD) to a standard curve.

Statistical Analysis

The influence of variance factors on the research parameters was determined using the SAS (2018) program. The means were statistically compared using the T-test, the ANOVA, or the least significant difference (LSD) test. The various types of sickle cell anemia were statistically evaluated using the chi-square test (0.05 and 0.01 probability). Using the ROC (Operating Characteristic) curve, the diagnostic specificity, sensitivity, and predictive value of Cystatin C, NGAL, KIM-1, β2-microglobulin, NAG, and Nephrin were estimated.

Results

Clinical characteristics of the Study Groups:

The results indicated that a difference in sickle cell anemiapatients and control according to age, BMI, sex, history of blood transfusions, Systolic BP, and Diastolic BP. Where the mean of age in steady state patients (8.5±0.32) year no significant different from the mean of age in vaso-occlusive crisis (VOC) patients(7.87±0.65) year and control (7.3±1.63). The percentage of female is higher than of male percentage in all study groups. The BMI mean in steady state patients (24.45±0.43) kg/m^2 no different from the mean of BMI in vaso-occlusive crisis (VOC) patients(24.6 ±0.54) kg/m^2 are lower than the BMI mean in control (25.54±0.62) kg/m^2. A larger percentage of steady state patients (80.6 %) were history of

blood transfusions (0-3) times in month compared to vaso-occlusive crisis (VOC) patients (45.9%). There were no significant changes in Systolic blood pressurebetween steady state (125.0± 0.16) and vaso-occlusive crisis (124.5± 0.27) compared to control group (115.0± 0.12), while diastolic blood pressure shown no significant different among three groups, as table (1).

Table 1: Indicated clinical characteristics of sickle cell anemia patients and controls.

Characteristics		Sickle Cell anemia patients		Control NO. (60)	T-test	P-value
		Steady state patients NO. (35)	vaso-occlusive crisis (VOC) patients No. (35)			
Age (year) Mean ± SE		8.5 ± 0.32	7.87 ±0.65	7.3 ±1.63	3.367	0.16 NS
BMI Mean ± SE		24.45±0.43	24.6±0.54	25.54 ±0.62	1.325	0.023*
Sex (M/F) No (%)		14/ 21 (40%) / (60%)	17/ 18 (48.5%) / (51.4%)	28/ 32 (46.6%)/(53.3%)	-	0.01 **/ 0.01 **
History of blood transfusions (n %)	0–3 months	80.6 %	45.9 %	-		0.0018 **
	> 3 months	19.4%	54.1%	-		0.001 **
Systolic BP, mm Hg		125.0± 0.16	124.5± 0.27	115.0± 0.12		0.0021 **
Diastolic BP, mm Hg		77.0± 0.22	75.5± 0.31	72.0± 0.18		0.058 NS

** (P≤0.01), * (P≤0.05), **NS:** Non-significant difference.

BMI: body mass index, **F:** Female, **M:** Male, **BP:** blood pressure.

Determination of hematological parameters in different groups

Hemoglobin, hematocrit, white blood cells, red blood cells, MCV, and platelets differ significantly between study groups (P≤0.01) in Table (2). There was significant decrease in mean of Hemoglobin and Hematocrit **%** in Steady state Patient (10.2±0.12ng/ml and 34.71±5.15%) and vaso-occlusive crisis groups (9.6±1.35ng/ml and 28.6±2.34) respectively, in contrast to the control group (13.4 ± 0.85 ng/ml and 42.6 ± 3.15%) respectively. Also, RBC had been significant decrease in Steady state Patient group (3.63±0.68) and vaso-occlusive crisis groups (3.14 ± 0.78)compared with control (5.01±0.58). While, WBC and PLT had been significant increase in Steady state Patient group (7.7 ± 1.23 and 238 ± 132) and vaso-occlusive **crisis** groups (11.5 ± 1.34 and 324 ± 112)compared with control (6.8±1.35 and 298.2± 110).

Table 2. Hematological characteristics of sickle cell anemia and the control group (Mean ± SD)

Parameters	Sickle Cell anemia patients		control	LSD value	P-value
	Steady state patients NO.(35)	vaso-occlusive crisis (VOC) patients (35)			
Hemoglobin (g/dl)	10.2 ± 0.12[a]	9.6±1.35[b]	13.4±0.85[c]	1.432 **	P≤0.01
Hematocrit (%)	34.71±5.15[a]	28.6±2.34[b]	42.6±3.15[c]	1.641**	P≤0.01
RBC ($\times 10^6$ cells/µL)	3.63±0.68[a]	3.14±0.78[a]	5.01±0.58[b]	4.121 *	P≤0.01
WBC count, $\times 10^3$/µL	7.7±1.23[a]	11.5±1.34[b]	6.8±1.35[c]	1.332 **	P≤0.01
PLT ($\times 10^3$ cells/µL)	238±132[a]	324±112[b]	298.2± 110[c]	1.641**	P≤0.01
Means having with the different letters in same row differed significantly. ** (P≤0.01).					

Comparison of renal function in sickle cell anemia:

Table (3) show a significant different in some renal function markers among study groups at (P≤0.01). Where proteinuria, urea, cystatin C, NGAL, KIM-1, NAG, β2-microglobulin, and Nephrin had been significant increase in Steady state Patients group (167.4 ±6.3,3.84±0.41, 1.4 ±0.12, 6.2±1.35, 3.79 ±3.21 , 4.3±1.2, and 4.5±0.3) respectively and vaso-occlusive crisis (VOC) (221±4.5, 4.3±1.4, 2.3±0.41, 10.35 ±0.21, 5.3±0.82, 7.2±1.6, and 6.8±0.8) respectively compared with control group (32±1.3, 3.6±0.78, 0.78±0.03, 3.8±1.03, 1.8±0.08, 3.14±0.7, and 2.12±0.24) respectively. Creatinin had been significant increase in vaso-occlusive crisis (VOC) (1.2±0.2) followed by steady state patients (0.94±0.1) compared with control group (0.9±0.07). While NAG had been significant increase in steady state patients (129±5.3) compared with vaso-occlusive crisis 83±4.81 and control72±4.62. $eGFR_{creat}$ had been significant increase in steady state patients 125.28±5.3, while significant decrease in vaso-occlusive crisis 110.8±3.65 compared with control 120.7±2.2.

Table 3: Comparison of renal function parameters in sickle cell anemia and control groups.

Parameters	**Sickle Cell anemia patients** (Mean ± SD)		Control	LSD- value
	Steady state Patients NO. (35)	**vaso-occlusive crisis (VOC) patients NO. (35)**		
Proteinuria Albumin to creatinin ratio (30-300) mg/g	167.4 ± 6.30[a]	221±4.5[b]	32±1.3[c]	0.924 **
Serum Urea (mmol/L) N.R(1.8-6.4)	3.84±0.41[a]	4.3±1.4[b]	3.6±0.78[c]	1.641**
Serum Creatinin (mg/dL) N.R. (0.6-1.3)	0.94 ± 0.1[a]	1.2 ± 0.2[a]	0.9±0.07[b]	4.121 *
Serum Cystatin C (0-20) ng/mL	1.4 ±0.12 [a]	2.3±0.41[b]	0.78±0.03[c]	2.463**
serum NGAL 0.16-10 ng/mL	6.2±1.35[a]	10.35 ±0.21[b]	3.8±1.03[c]	1.332 **
serum KIM-1 0.32-20 ng/mL	3.79 ±0.21 [a]	5.3±0.82[ab]	1.8±0.08[c]	1.687**
Serum β2-microglobulin 0.32-20 ng/mL	4.3±1.2[a]	7.2±1.6[b]	3.14±0.7[c]	1.27**
NAG 1.57-100 ng/mL ng/mL	129±5.3[a]	83±4.81[b]	72±4.62[c]	0.973 **
Nephrin 0.16–10 ng/mL	4.5±0.3[a]	6.8±0.8[b]	2.12±0.24[c]	2.381**
$eGFR_{creat}$ (mL/min/1.73m^2)	125.28±5.3[a]	110.8±3.65[ab]	120.7±2.2[c]	0.604 **
Means having with the different letters in same row differed significantly. *(P≤0.05), ** (P≤0.01).				

Determination of the diagnostic sensitivity, specificity, Negative predictive value, Positive predictive value, and Accuracy of study parameters in study groups.

The ROC analysis of Cystatin C, NGAL, KIM-1, β2-microglobulin, NAG, and Nephrin concentrations in sickle cell anemia patients and controls is shown in Table 7 and Figure 1. The pest cut-off value of Cystatin C was 1.1 ng/ml. Cystatin C levels greater than 1.1 demonstrated a specificity 87% and a sensitivity 99% for sickle cell nephropathy diagnosis. The pest NGAL cut-off value was 5.6 ng/ml. NGAL concentration greater than

5.6 demonstrated a specificity 78% and a sensitivity 91 % for sickle cell nephropathy diagnosis. The pest KIM-1 cut-off value was 9 ng/ml. KIM-1 concentrations greater than 9 ng/ml estimated a sensitivity 95% and specificity 71% for sickle cell nephropathy diagnosis. The pest β2-microglobulin cut-off value was 10.8 ng/ml. A β2-microglobulin concentration greater than 10.8 was estimated to have a specificity 72% and a sensitivity 84% for sickle cell nephropathy diagnosis. The pest NAG cut-off value was 88 ng/ml. NAG concentration greater than 88 estimated a specificity 86% and sensitivity 92% for sickle cell nephropathy diagnosis. The pest Nephrin cut-off value was 6 ng/ml. Nephrin levels greater than 6 ng/ml estimated a sensitivity 97 % and specificity 91% for sickle cell nephropathy diagnosis.

Table 4: Sensitivity, Specificity, PPV, NPV, and Accuracy for study parameters in sickle cell anemia.

Parameter	Cut-off	Specificity	Sensitivity	NPV	PPV	Accuracy
Serum Cystatin C	1.1 ng/ml	87%	99%	96%	98%	97.7%
Serum NGAL	5.6 ng/ml	78%	91%	88%	92%	86 %
Serum KIM-1	9 ng/ml	71%	95%	94 %	95 %	85 %
Serum β2-microglobulin	10.8 ng/mL	72%	84%	95%	98%	97%
Serum NAG	88 ng/mL	86%	92%	93%	97%	96%
Nephrin	6 ng/mL	91%	97%	92 %	98%	91%

***PPV**= positive predictive value, **NPV**= Negative predictive value

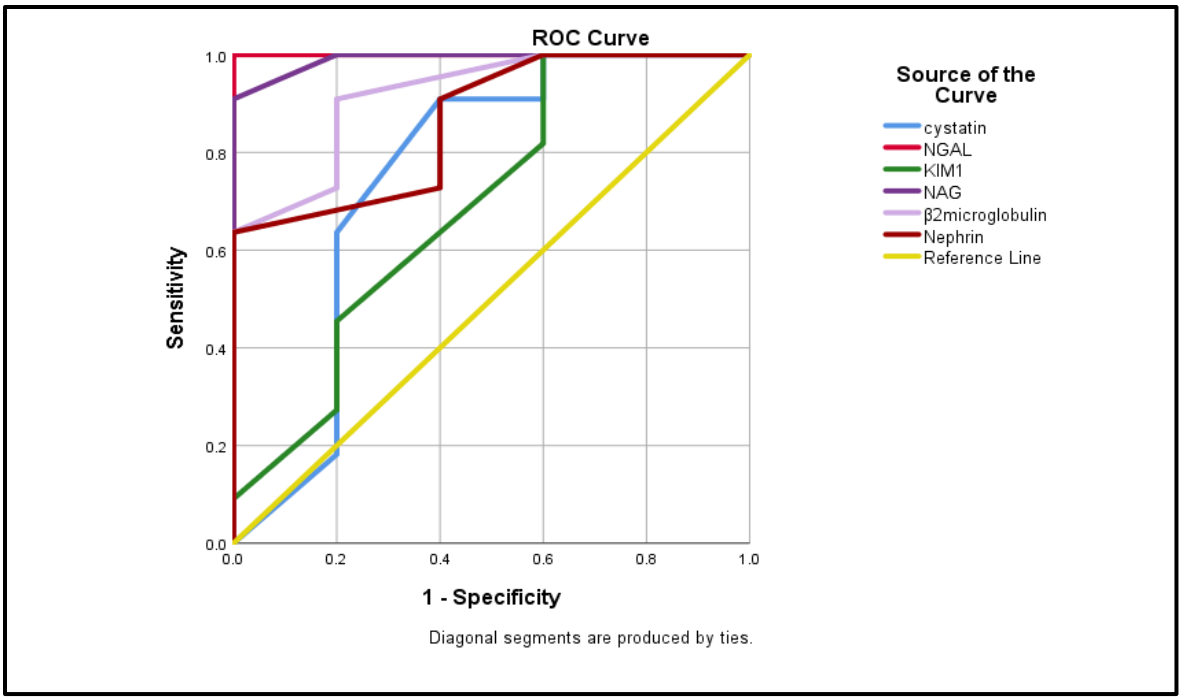

Figure 1. Cystatin C, NGAL, KIM-1, β2-microglobulin, NAG, and Nephrin ROC curve analysis for cystatin C level (sickle cell anemia patients versus controls). Cystatin ROC AUC= 0.755; 95% CI, 0.437–1.000. NGAL ROC AUC= 1.000; 95% CI, 1.000 -1.000. KIM1 ROC AUC = .691; 95% CI, .384-0.998. NAG ROC AUC = .991; 95% CI, .955-1.000. β2-microglobulin ROC AUC = .918; 95% CI, .775-1.000. Nephrin ROC AUC = .864; 95% CI, .677-1.000.

Discussion

In present study indicates a significant different in hematological parameters in sickle cell anemia patients.Hb concentration and PCV(%) were significantly decrease in both patient groups relative to controls in table (2). These results align with [1] that reporting significant decline in Hb and PCV during crisis episodes, reflecting the acute worsening of anemia.These are related to the pathophysiology of sickle cell anemia, where persistent anemia is caused by poor erythropoiesis and prolonged hemolysis [4]. Increased hemolysis, arterial blockage, and RBC sequestration in the spleen and other organs are the main causes of the reduction during VOC [20];[21].

Similarly, RBC counts were significantly lower in sickle cell patients. The reduced RBC numbers highlight the chronic hemolytic nature of the disease, where the lifespan of sickled erythrocytes is shortened 10–20 days compared to 120 days in normal RBCs[22].In contrast, WBC counts were significantly elevated in both steady state and VOC groups. The well-known feature of leukocytosis in sickle cell disease is linked to increased cytokine production, bone marrow hyperactivity, and chronic inflammation [19]. Through increased leukocyte adherence to the endothelium and interaction with sickled red blood cells, higher WBCs are believed to have a role in the pathophysiology of vascular occlusion during VOC [23]. This implies that the WBC

count contributes to the severity of a crisis in addition to being a sign of inflammation [22].

GFR markers (serum urea, creatinine, and eGFRcreat) are less helpful than more recent sickle cell nephropathy markers (cystatin C, urine NGAL, KIM-1, β2-microglobulin, NAG, and nephrin) in identifying renal involvement during VOC, according to our analysis of the performance characteristics of serum nephropathy biomarkers [24].

Proteinuria was increased significantly in steady state patients and VOC. This result agrees with [1], who discovered that 32% of participants in VOC and 17.1% of those in steady state had microalbuminuria. This is consistent with the well-documented association between sickle cell nephropathy and glomerular damage, particularly glomerular hyperfiltration, podocyte injury, and progressive albuminuria [25]. In sickle cell patients, elevated proteinuria has been associated with a higher risk of chronic kidney disease (CKD) and is an early indicator of nephropathy. Gross proteinuria (macroalbuminuria) was the metric examined in the few other studies in our region that addressed SCN; however, more sensitive biomarkers such as urine NGAL and the microalbumin:creatinine ratio might better reveal renal failure at an earlier stage [1; 24].

It is highly likely that, in the absence of severe glomerular injury that impairs GFR, renal tubular damage may occur prior to the development of microalbuminuria [25]. The pathophysiologic alterations in SCN start at an early age with progressive age-dependent worsening of renal function due to recurrent glomerular and tubulo-interstitial compartment damage [26]. Because serum creatinine levels vary depending on factors such as muscle mass, gender, race, medicines, and concomitant diseases, they are not a reliable indicator [27]. Serum creatinine levels in SCD patients are low because of increased urine output; as a result, they only rise in the latter stages of SCN [1]. SCA patients also have higher than normal GFR and renal blood flow; therefore, urinary creatinine clearance is high [28]. Our research demonstrates the varying use of microalbumin excretion and creatinine level in serum, two conventional indicators of SCN, in identifying renal involvement during VOC. This supports the hypothesis that early sickle nephropathy is characterized by hyperfiltration, which predisposes to long-term glomerular damage [24].

Cystatin C was significantly elevated in both patient groups. This reinforces the evidence that glomerular dysfunction is not fully captured by serum creatinine alone, and cystatin C may provide earlier detection of subclinical renal impairment in sickle cell anemia [29]. Cystatin C showed the best performance, with an optimal cut-off value of 10 ng/ml, yielding 100% sensitivity and 99% specificity. Cystatin C is a better indicator of GFR in SCD, according to earlier research [30]. This result underscores the superiority of Cystatin-C over creatinine as a marker of glomerular filtration rate (GFR), particularly in conditions such as sickle cell disease, where muscle mass and creatinine production are often altered [27].

The use of this biomarker as an indication of renal involvement during VOC is supported by noticeably greater urine NGAL in VOC patients as compared with patients with

steady state (Table 3). Ferritin and urine NGAL were discovered to be correlated in a prior study [1]. They concluded that the correlations between plasma and urine NGAL and hematological markers in VOC indicate renal involvement in SCD patients with seemingly normal renal function [31]. Inflammatory processes produce NGAL, a hallmark of neutrophil activity, which is then freely filtered in the glomeruli and reabsorbed by endocytosis in the proximal tubule. NGAL is a useful early indicator of acute kidney damage since it is known to increase quickly in response to tubular injury [13]. The elevated levels observed in sickle cell patients, especially during vaso-occlusive crisis, reflect ongoing tubular stress and injury due to ischemia-reperfusion events. Thus, NGAL may be particularly valuable for identifying patients at risk of acute kidney injury superimposed on chronic sickle nephropathy [32].

KIM-1, another marker of tubular injury, also displayed excellent ROC performance [33]. Its high specificity and sensitivity confirm its role as a reliable biomarker for detecting tubular epithelial cell injury [14]. Elevated KIM-1 in sickle cell patients may reflect both acute and chronic tubular damage due to hemolysis, oxidative stress, and microvascular occlusion [12]. Similarly, β2-microglobulin demonstrated very strong diagnostic accuracy. As a protein with low molecular weight that the glomerulus filters and the proximal tubules reabsorb, its elevation suggests combined glomerular and tubular dysfunction [30].

Also, Nephrin was significantly elevated in both steady state and VOC groups. This suggests podocyte injury and glomerular barrier dysfunction as early contributors to proteinuria development in sickle cell nephropathy. Nephrin indicates early glomerulopathy; useful in SCD patients [34].NAG showed good sensitivity and specificity, although slightly lower compared to Cystatin C and β2-microglobulin. Interestingly, higher NAG levels in steady state patients compared to VOC suggest that it may be more reflective of chronic, low-grade tubular damage rather than acute injury [16]. Our findings raise the question of the ideal marker that is frequently employed to detect renal dysfunction during sickle cell anemia. We recommended a suitable cut-off value and determined the optimal marker for screening for renal involvement using ROC analysis. On the basis of these findings, Cystatin C, urine NGAL, and KIM-1 all exhibited high diagnostic accuracy in distinguishing sickle cell anemia patients from healthy controls. Early diagnosis and appropriate treatment would decrease mortality and morbidity in patients with SCD [7].In the present study, according to conventional indicators, the research participants' renal function was normal. Particularly during VOC, this patient group would not have been thought to have renal involvement. However, the existence of renal involvement may be indicated by the more recent indicators of acute kidney damage, particularly during VOC.

Conclusion

Based on these findings, we identified a better biomarker for routine screening to detect renal involvement during sickle cell anemia. Cystatin C, NGAL, and KIM-1 all

exhibited high diagnostic accuracy in distinguishing patients with sickle cell anemia from healthy controls.

DATA AVAILABILITY

Study data will be available upon request to the corresponding author.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this article.

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الكشف المبكر عن اعتلال الكلى لدى الأطفال العراقيين المصابين بفقر الدم المنجلي باستخدام مؤشرات فسيولوجية حديثة.

الخلاصة

هدفت هذه الدراسة إلى تقدير الخصائص التشخيصية والتنبؤية لمؤشرات اعتلال الكلى (سيستاتين سي، وليبوكالين المرتبط بالجيلاتيناز العدلي (NGAL)، وبيتا-2 ميكروغلوبولين، وإن-أسيتيل بيتا-دي-غلوكوزامينيداز (NAG)، ونيفرين، وجزيء إصابة الكلى 1 (KIM-1) في الحالة المستقرة وأزمة انسداد الأوعية الدموية المصاحبة لفقر الدم المنجلي. شملت الدراسة 70 طفلاً مصاباً بفقر الدم المنجلي تتراوح أعمارهم بين 3 و18 عاماً، و60 طفلاً سليماً تتراوح أعمارهم بين 3 و18 عاماً. قُسِّم مرضى فقر الدم المنجلي إلى مجموعتين: 35 مريضاً في الحالة المستقرة، و35 مريضاً في أزمة انسداد الأوعية الدموية. استُخدم اختبار المقايسة المناعية المرتبطة بالإنزيم لتقدير مستويات السيستاتين C، وNGAL، وبيتا-2 ميكروغلوبولين، وNAG، والنيفرين، وKIM-1 لدى المرضى ومجموعة السيطرة.

أظهرت النتائج ارتفاعًا معنوياً في مستويات السيستاتين C، وNGAL، وKIM-1، وNAG، وβ2-ميكروغلوبولين، والنيفرين في مجموعة المرضى ذوي الحالة المستقرة (3.84 ± 2.41، 1.35 ± 0.21، 6.2 ±3.79، 5.3 ± 129, 1.2±4.3, و0.3 ±4.5) على التوالي ومجموعة مرضى انسداد الأوعية الدموية (0.41± 2.3, 0.21 ±10.35, 0.82±5.3, 4.81±83, 1.6 ± 7.2, و0.8± 6.8) على التوالي مقارنةً بمجموعة السيطرة (0.03±0.78, 1.03 ± 3.8, 0.08±1.8, 4.62±72, 0.7± 3.14, و0.24 ±2.12) على التوالي. بناءً على هذه النتائج، حددنا أفضل مؤشرٍ للفحص الروتيني للكشف عن إصابة الكلى أثناء فقر الدم المنجلي. أظهر كلٌ من السيستاتين C، وNGAL، وKIM-1 دقةً تشخيصيةً عاليةً في التمييز بين مرضى فقر الدم المنجلي والأفراد الأصحاء.

الكلمات المفتاحية: اعتلال الكلى، فقر الدم المنجلي، المعايير الفسيولوجية