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ORIGINAL ARTICLE

Albuminuria correlates with hemolysis and NAG and KIM-1 in patients with sickle cell anemia

Dima Hamideh · Vimal Raj · Thomas Harrington · Hua Li · Emilio Margolles · Folasade Amole · Monica Garcia-Buitrago · Phillip Ruiz · Gaston Zilleruelo · Ofelia Alvarez

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Abstract
Background Although hyperfiltration and albuminuria are common pathological conditions, kidney injury (KI) biomarkers have been seldom studied in individuals with sickle cell anemia (SCA).
Methods We undertook a cross-sectional assessment of urine KI biomarkers in children and adults with SCA with and without albuminuria and a normal estimated glomerular filtration rate (eGFR). Albumin, KI molecule 1 (KIM-1), *N*-acetyl-ß-D-glucosaminidase (NAG), endothelin-1 and transforming growth factor-β_1 (TGF-β_1) were measured. Assays were normalized by urine creatinine. Urine intracellular hemosiderin and serum lactate dehydrogenase (LDH) were assessed as markers of hemolysis. Albuminuria was associated to the biomarkers by Pearson and Spearman correlation coefficients. Differences between the albuminuria (yes, no) groups were assessed by the *t* test.
Results Nineteen patients with albuminuria (mean urine albumin/creatinine 527.14±1070 mg/g, range 38.3-–190 mg/g) and 19 patients without albuminuria (mean urine albumin/creatinine 15.93±5.17 mg/g, range 7.9–28.4 mg/g) were studied. The age range for the whole group was 11–48 years, and 47 % were males. Patients with albuminuria were older, had lower hematocrit, were more likely to test positive for urine hemosiderin and had a higher KIM-1 (P=0.0035) and NAG/creatinine ratios (P=0.0062). Urine hemosiderin strongly correlated to a higher LDH level (P<0.001).
Conclusions Despite a normal or increased eGFR, KI biomarkers were detected in the urine of individuals with SCA. NAG, KIM-1 and urine hemosiderin correlated with the presence of albuminuria.

Keywords Albuminuria · Biomarkers · Hemosiderin · Nephropathy · Proteinuria · Sickle cell anemia

D. Hamideh · O. Alvarez (✉)
Division of Pediatric Hematology (D-820), University of Miami, PO Box 016960, Miami, FL 33101, USA
e-mail: oalvarez2@med.miami.edu

V. Raj · G. Zilleruelo
Division of Pediatric Nephrology, University of Miami, Miami, FL, USA

T. Harrington
Division of Hematology, University of Miami, Miami, FL, USA

H. Li
Department of Public Health Sciences, University of Miami, Miami, FL, USA

E. Margolles · F. Amole · M. Garcia-Buitrago · P. Ruiz
Department of Pathology and Laboratory Medicine, University of Miami, Miami, FL, USA

P. Ruiz
Department of Surgery, University of Miami, Miami, FL, USA

Introduction

Sickle cell disease (SCD) refers to a group of autosomal recessive hemolytic anemias in which the erythrocytes contain a predominance of sickle hemoglobin (HbS) due to the inheritance of a β-globin mutation. The most severe SCD genotype is sickle cell anemia (Hb SS, SCA). The pathogenesis of sickle cell disease involves hemolysis and repeated episodes of ischemia and reperfusion in all organs, including the kidney [1–4]. Thus, early recognition of developing organ damage is imperative in order to institute specific therapeutics which might modify clinical course.

Sickle cell nephropathy (SCN) is an important cause of mortality in patients with SCA [5–7]. Chronic sickling and ischemia promote kidney injury (KI), such as urine concentration defects, glomerular hyperfiltration and glomerular enlargement and sclerosis [5, 8]. Renal injury is not easily

identifiable at early stages of SCN, since standard renal function tests, such as serum creatinine and creatinine-based estimation of glomerular filtration rate (eGFR) become abnormal only when renal damage has become extensive and largely irreversible [9, 10]. Currently, the most reliable sign of SCN is albuminuria, which occurs in about 19 % of children aged 10 years and older [11] and 68 % of adults with SCA [12], although only a proportion of patients with albuminuria progress to more advanced stages of chronic kidney disease [5, 9]. Recently, there has been a great surge of interest in identifying novel biomarkers that can be easily detected in the urine and which can be used to diagnose renal injury at the earliest stages [3–18]. Such biomarkers have been seldom studied in SCD [19–21]. Therefore, the purpose of this study was to evaluate the presence of urine KI biomarkers in patients with SCA and to correlate these markers with albuminuria, as albuminuria is a common finding signaling KI. We selected several markers evaluated in a previous study [KI molecule 1 (KIM-1), *N*-acetyl-ß-D-glucosaminidase (NAG), transforming growth factor-β_1 (TGF-β_1)] [19] and two other biomarkers which could be more specific for sickle cell disease [endothelin-1 (ET-1) and urine hemosiderin]. Briefly, NAG, a tubular lysosomal brush border enzyme, is a biomarker of proximal tubule injury, which has been observed in diabetic patients without albuminuria and to further increase in patients with albuminuria [22]. NAG has also been reported in patients with SCD and albuminuria [19]. KIM-1 is a trans-membrane protein that is not expressed in the normal kidney, but which is specifically expressed in injured proximal tubular cells [23]. TGF-β_1 is the main modulator of the healing process after tissue injury and increases with renal fibrosis [19]; therefore its detection may be important for chronic kidney disease. ET-1 is a 21-amino acid peptide implicated in the development and progression of chronic kidney disease. Urinary ET-1 excretion increases twofold in SCD patients compared to healthy controls [24]. Furthermore, as hemolysis is an integral part of the pathophysiology of SCA, we decided to evaluate for the presence of urinary hemosiderin and correlate this finding with albuminuria and the other KI biomarkers.

Methods

Subjects

All procedures were done in accordance with ethical standards and according to the Declaration of Helsinki of 1975, as revised in 2000. The University of Miami Institutional Review Board approved the study. Subjects aged 10 years and older were eligible to participate in this cross-sectional study, regardless of whether they tested positive or negative for albuminuria. This age choice was based on our previous finding that children aged 10 years and older will have a relatively higher prevalence of albuminuria than younger children [11]. All adult subjects and parents gave their informed consent, and children aged 10–17 years gave their assent when applicable, prior to participation. Patients with pre-existing non-sickle cell kidney disease (i.e. diabetes, hypertension, lupus nephritis) were not eligible to participate in the study. All patients were at baseline status (no acute sickle event within 4 weeks prior to sample collection).

Laboratory assessments and definitions

Concurrent urine and blood samples were collected from the participants during their routine clinical visit for laboratory testing, including hemoglobin, reticulocyte count, lactate dehydrogenase (LDH), serum creatinine, serum cystatin C, urine protein and urine albumin/creatinine ratio. Urine samples were collected for KI biomarkers and stored at −80 °C until analysis. We considered the urine albumin/creatinine ratio to be elevated at ≥30 mg/g creatinine and the urine protein/creatinine ratio to be elevated at ≥0.2 mg/mg.

Random urine protein, albumin and creatinine were assayed by light spectrophotometry; the results were confirmed in a first morning urine collection to evaluate whether albuminuria was orthostatic. In addition, urine osmolality was obtained from the first morning urine after overnight fluid deprivation. The GFR was estimated from serum creatinine by using the bedside Schwartz formula for children [25, 26] and the abbreviated Modification of Diet in Renal Disease formula for adults [27]. The GFR was also estimated from serum cystatin C measurements by using the Filler formula [28].

Determination of urine KI biomarkers

The concentration of NAG in the urine was measured by spectrophotometry using the assay NAG VRA-GlcNAc test kit (Praill Price Richardson Diagnostics Ltd, London, UK) according to the manufacturer's recommendations.

The concentrations of TGF-β_1, KIM-1 and ET-1 were determined in a Bioplex 200 Luminex system (Bio-Rad, Hercules, CA) using the multiplex bead-based technology kits Milliplex MAP TGF-β_1—Single Plex—Immunology Assay, Milliplex MAP Human Kidney Toxicity Magnetic Bead Panel 1—Toxicity Multiplex Assay (customized for KIM-1 as Single Plex) and Multiplex MAP Human Angiogenesis/Growth Factor Magnetic Bead Panel—Cancer Multiplex Assay (customized for endothelin-1 as Single Plex), respectively, following the manufacturer's recommendations (EMD Millipore Corp., Billerica, MA).

All urine biomarkers were expressed as a ratio of urine creatinine. Published biomarker data from healthy subjects were used as reference: ET-1, 7.22±2.17 pg/mmol creatinine [24]; NAG, <2 U/L; KIM-1, 0.228±0.188 ng/mg creatinine,[29]; TGF-β_1, 26.6±6.3 pg/mL [30].

Urine hemosiderin

Urine was centrifuged and the sediment stained with Perl's Prussian blue. Slides were microscopically examined to assess for the absence or presence of hemosiderin-laden epithelial cells [31, 32].

Statistical analysis

Descriptive analyses were produced for each set of the study measurements. Comparisons between patients with and without albuminuria were conducted using chi-square tests for categorical variables, and *t* tests and Wilcoxon Rank-Sum tests for normally and non-normally distributed continuous variables, respectively. The variables we explored were patient's age, gender, use of hydroxyurea (hydroxycarbamide) or chronic transfusions, hemoglobin, hematocrit, reticulocyte count, LDH, serum creatinine, serum cystatin C, eGFR, urine protein/creatinine ratio, urine albumin/creatinine ratio, urine osmolality and urine biomarkers. The correlations of albuminuria with the urine biomarkers were analyzed by Pearson and Spearman correlation coefficients. To formally estimate and test the association between groups and continuous biomarker outcomes, we used a linear regression model while adjusting for other variables, such as age. All tests were two-sided with a significance level of 0.05. All statistical analyses were conducted using Statistical Analysis Software (SAS) ver. 9.2 (SAS Institute, Cary, NC).

Results

Nineteen subjects with SCA and known albuminuria or proteinuria and 19 subjects without albuminuria or proteinuria were enrolled. All patients were homozygous for Hb S. The patient cohort comprised 18 males and 20 females, with ages ranging from 11 to 48 (mean age 19.74) years.

Clinical characteristics and laboratory results in patients with and without albuminuria

The clinical characteristics and laboratory data on hematological and renal parameters of the patients with SCA with albuminuria versus those without albuminuria are presented in Table 1. Nineteen patients had albuminuria (mean urine albumin/creatinine 527.14±1,070 mg/g, median 140.85 mg/g, range 38.3-4190 mg/g), and 19 patients did not have albuminuria (mean urine albumin/creatinine 15.93±5.17 mg/g, median 15.92 mg/g, range 7.9-28.4 mg/g). The patients with albuminuria were significantly older than those without albuminuria (P=0.0088). There were no significant differences in the albuminuria status between the random and the first morning urine samples. Only one subject of the 17 without albuminuria in the random urine sample had albuminuria in the first morning specimen, and two subjects of the 13 with albuminuria in the random urine specimen did not have it in the first morning urine sample.

There was no significant difference by gender for the presence of albuminuria, with ten males with albuminuria and eight males without albuminuria, respectively (P=0.51). Most patients in the study were either on hydroxyurea (24/38 patients) or chronic transfusions (11/38 patients), with no significant association of either treatment to the presence or absence of albuminuria. Ten subjects [4 in the albuminuria group (21 %) and 6 in no albuminuria group (31.6 %)] were taking deferasirox for iron chelation; there was no statistical difference between groups. One patient on chronic transfusion and one patient on no therapy had nephrotic-range proteinuria (2,685 and 4,190 mg/g, respectively). Eight patients were receiving either an angiotensin-converting enzyme inhibitor (N=5) or angiotensin receptor blocker (N=3). Due to the cross-sectional nature of this study, two of the eight patients receiving angiotensin blockade did not have albuminuria at the time of the sample, so that they were analyzed in the group without albuminuria.

The mean serum creatinine, serum cystatin C and GFR derived from serum creatinine and cystatin C were similar in patients with or without albuminuria. Patients with albuminuria had a lower mean hematocrit, but there were no statistical differences for hemoglobin, reticulocyte count or LDH between groups. Impaired urine concentrating ability was observed in the majority of patients. All patients in the albuminuria group who had urine osmolality tested (N=13) had an osmolality of <500 mOsm/kg H_2O whereas four of the 18 patients (22 %) in the group without albuminuria had a urine osmolality of >500 mOsm/kg H_2O, with one patient having the maximum observed osmolality of 632 mOsm/kg H_2O.

Urine KI biomarkers and albuminuria

The data on KI biomarkers are presented in Tables 2 and 3. In the sickle cell patients, urinary NAG activity was elevated above 2U/L [19, 20], a previously described cutoff, in 32 of the 38 (84.2 %) patients; 94.7 % (18/19) of the patients with albuminuria and 73.7 % (14/19) of those without albuminuria had elevated NAG. NAG activity was significantly increased in patients with albuminuria (P=0.0062).

Of the 38 patients, 24 (63.1 %) had elevated KIM-1 levels compared to normative data [29]; 17 of 19 patients (89.5 %) with albuminuria had elevated KIM-1 levels, but only seven of the 19 (36.8 %) patients without albuminuria had increased KIM-1 levels. Similarly, when urinary KIM-1 levels were compared between the two groups, patients without albuminuria had slightly lower levels, whereas patients with albuminuria had significantly increased levels (P=0.004).

Table 1 Age, clinical and laboratory parameters in patients with and without albuminuria

Parameters	Albuminuria group		No albuminuria group		*P* value
	Mean±SD	Minimum, maximum	Mean±SD	Minimum, maximum	
Age (years)	23.32±10.21	14, 48	16.16±3.79	11, 26	0.0088
Systolic blood pressure (mmHg)	115.58±17.39	87, 162	120.05±12.92	103, 152	0.3739
Diastolic blood pressure (mm Hg)	69.00±11.36	52, 96	65.74±11.45	46, 93	0.3837
Body mass index	20.34±3.27	15.04, 27.28	20.73±3.09	15.37, 26.12	0.7071
Hemoglobin (g/dL)	8.15±0.91	6.6, 9.5	8.79±1.20	7.1, 12.3	0.0733
Hematocrit (%)	23.99±3.10	19.4, 28.8	26.20±3.29	21.1, 35.3	0.0406
Reticulocytes (%)	14.64±6.47	2.9, 25.0	12.84±6.04	7.0, 30.0	0.3825
Lactate dehydrogenase (U/L)	1,243.67±468.39	504, 2105	1,168.94±508.93	604, 2,697	0.6496
Serum creatinine (mg/dL)	0.59±0.27	0.4, 0.70	0.50±0.11	0.39, 0.70	0.1701
Creatinine-eGFR (mL/min/1.73 m^2)	131.62±40.55	48.73, 223.02	139.03±24.70	104.92, 174.49	0.5020
Serum cystatin C (mg/L)	0.69±0.19	0.5, 1.06	0.68±0.14	0.5, 1.10	0.8853
Cystatin eGFR (mL/min/1.73 m^2)	134.73±37.97	71.76, 185.28	131.49±27.70	68.48, 185.28	0.7676
Urine osmolality(mOsm/kg)	387.77±73.08	192, 449	448.17±99.94	243, 632	0.0749
Urine protein/creatinine	0.89±1.60	0.19, 6.5	0.15±0.06	0.07, 0.3	0.0599
Random urine A/C[a]	140.85, 229.2	38.3, 4,190.0	15.92, 7.58	7.91, 28.46	<0.0001
First morning urine A/C[a]	69.28, 44.64	12.38, 117.65	14.48, 8.67	6.69, 285.71	0.0004

A/C, Albumin to creatinine ratio; eGFR, estimated glomerular filtration rate; SD, standard deviation

[a] Values are presented as the median and interquartile difference. Wilcoxon rank-sum test was used for comparison

Because the participants with albuminuria were significantly older than those without albuminuria, we performed regression analysis to control for age. There was no statistically significant difference after controlling for age in NAG levels (P=0.0686); however, KIM levels were significantly higher in the patients with albuminuria (P=0.0155). The eight patients who were on angiotensin blockade had elevated NAG, regardless of whether they were positive (N=6) or negative (N=2) for albuminuria at the time of sampling. In contrast, the two patients who were negative for albuminuria had a normal KIM-1/creatinine ratio, whereas those with albuminuria remained with elevated KIM-1 ratios.

Urine TGF-β_1 was detected at low levels in only 51 % of the samples, making the interpretation difficult. We did not detect an association between albuminuria and urine ET-1. The presence of intracellular hemosiderin was significantly higher in those patients with albuminuria. Whereas 12 of 19 (63 %) patients with albuminuria were positive for urine hemosiderin, only three of 19 (18 %) patients without albuminuria tested positive (P=0.0057).

Urine KI biomarkers and kidney function and hemolysis

A strong association was found between albuminuria and the hemolytic markers LDH (P<0.001) and hemosiderin (P<0.008). Urine hemosiderin strongly correlated to LDH (P<0.001), and urine ET-1 was inversely correlated with hemoglobin and hematocrit by the both Pearson and Spearman coefficients (P=0.01). Urine osmolality positively correlated with hemoglobin (P=0.0011) and hematocrit (P=0.002).

Table 2 Urine kidney injury biomarkers in patients with and without albuminuria

Biomarkers	Albuminuria group (N=19)		No albuminuria group (N=19)		*P* value
	Median, IQR	Minimum, maximum	Median, IQR	Minimum, maximum	
KIM-1 (pg/mL)	608.10, 311.3	32.20, 4553.20	200, 389.7	36.90, 990.30	0.0024
NAG (U/L)	5.36, 12.04	1.66, 45.31	2.57, 3.99	0.88, 12.95	0.0055
ET-1 (pg/mL)	14.50, 2.4	13.80, 150.60	14.50, 17.2	4.20, 61.90	0.3729
TGF-β1 (pg/mL)	17.91, 15.65	14.39, 1529.45	12.83, 14.56	11.26, 35.01	0.0733

KIM-1, Kidney injury molecule 1; NAG, *N*-acetyl-ß-D-glucosaminidase; ET-1, endothelin-1; TGF-β_1, transforming growth factor-β_1; IQR, interquartile range

Table 3 Kidney injury biomarkers corrected for urine creatinine

Biomarkers	Albuminuria group (N=19)		No albuminuria group (N=19)		P value
	Mean±SD	Minimum, maximum	Mean±SD	Minimum, maximum	
KIM-1/creatinine (ng/mg)	0.93±0.73	0.01,3.03	0.35±0.31	0.0,1.35	0.0035
NAG/creatinine (U/g)	10.33±8.25	1.86,24.75	4.26±2.97	1.08,12.85	0.0062
ET-1/creatinine (pg/mmol)	3.33±2.96	0.90,12.81	2.76±1.94	0.46,6.95	0.4916
TGF-β1/creatinine (ng/g)	0.22±0.87	0.00,3.82	0.01±0.02	0.00,0.06	0.3223

KIM-1, Kidney injury molecule 1; NAG, *N*-acetyl-ß-D-glucosaminidase; ET-1, endothelin-1; TGF-β_1, transforming growth factor-β_1; SD, standard deviation

NAG strongly correlated with age, albumin/creatinine ratio, urine protein/creatinine ratio, and KIM-1. In general, urine KIM-1, NAG, ET-1 or TGF-β_1 did not correlate to serum creatinine, serum cystatin C or their corresponding estimated GFR. All but three patients had creatinine-based GFR at ≥90 ml/m^2/1.73.

Discussion

Sickle cell nephropathy is one of the main chronic complications of SCD that begins in childhood and may progress to overt renal failure. Genetic factors, severity of anemia and overall disease severity are risk factors for SCN [6, 33, 34]. Data from the natural history study, the Cooperative Study of Sickle Cell Disease, revealed that almost 9 % of SCD patients died from overt renal failure [7]. In the University of Southern California School of Medicine cohort, SCD patients with end-stage renal disease (ESRD) survived only 4 years after the diagnosis of renal failure [6], and the median survival among patients with and without renal failure was 29 and 51 years, respectively. Even with dialysis or kidney transplantation, the survival of patients with SCD and renal failure is worse than that of non-SCD patients with renal failure [35].

The pathogenesis of SCN is complex and involves several steps that have been previously reviewed [5, 10, 11, 36]. Among these steps, glomeruli, renal tubules and vasa recta are damaged by ischemia–reperfusion events caused by intramedullary sickling. Vasodilating agents like prostaglandins and nitric oxide increase, thereby augmenting the blood flow to the remaining glomeruli with consequent hyperfiltration and glomerular hypertrophy. Eventually, intraglomerular hypertension leads to impaired filtration capacity and the loss of glomerular permselectivity to macromolecules and albuminuria. In addition, enhanced tubular epithelial endocytosis of proteins contributes to tubulointerstitial injury and fibrosis. Recently, hemolysis has been thought to contribute to vasodilation and inflammation [4].

There is an unmet need for highly sensitive biomarkers that could lead to the early detection of SCN. It is estimated that 4–18 % of patients with SCA will eventually progress to ESRD [6, 7]. Serum creatinine tends to be low in SCD patients due to supra-normal creatinine excretion in the urine; therefore, serum creatinine rises only in the late stages of SCN. More recently, serum cystatin C, a non-glycosylated low-molecular-weight basic protein that inhibits cysteine proteases, has been found to be a reliable surrogate of GFR. In contrast to creatinine, cystatin C is not secreted by the kidney [37–39], and serum cystatin C may become elevated at an earlier stage of kidney disease when compared to serum creatinine [10]. However, even earlier markers are desirable.

Considered the best predictor of progression to ESRD, microalbuminuria signals stage 1 chronic kidney disease, even without decreased GFR in certain patient populations. Among patients with SCA the prevalence of albuminuria is 20 % during the first and second decades of life [11], increasing during adulthood to >60 % [12]. However, many patients who have microalbuminuria will not progress at least in the short-term [40]; thus, it would be important to determine specific biomarkers for kidney disease progression so that individualized care could be provided.

Multiple biomarkers in serum and urine that represent different mechanisms or structural damage have been studied, based on whether they are classified as markers of glomerular injury, tubular injury, oxidative stress, inflammation and endothelial damage. NAG, a tubular lysosomal brush border enzyme, is a biomarker of proximal tubule injury. Urine NAG increases by ninefold in normoalbuminuric patients with diabetes compared to controls [22] and increases yet further with the development and progression of albuminuria. Furthermore, regression of albuminuria is associated with a highly significant reduction in urine NAG excretion in type 1 diabetic patients. In our study, urine NAG activity was elevated in most patients with SCA, even in the non-albuminuria group. When compared between groups with or without albuminuria, urine NAG activity was significantly increased in patients with albuminuria (P=0.0062). This finding corroborates the results of Sundaram et al. [19], who reported that most patients with and without albuminuria had elevated urine NAG activity.

KIM-1 is another biomarker that is associated with proximal tubule injury and acute and chronic kidney disease.

KIM-1 is a trans-membrane protein that is not expressed in the normal kidney, but is specifically expressed in injured proximal tubular cells. Urine KIM-1 levels are closely related to tissue KIM-1 levels and correlate with the severity of renal damage. Quantification of urine KIM-1 is a sensitive method for the evaluation of kidney injury and even for monitoring the impact of therapy on KI [23]. KIM-1 was detectable in all our SCA patients. When urine KIM-1 levels were compared between the two groups, the lowest levels were detected in patients without albuminuria; in contrast, patients with albuminuria had significantly increased levels (P=0.004). Again, this finding confirms the results of Sundaram et al. [19]. Based on the presence of elevated urine NAG and KIM-1, we can postulate that these tubular injury biomarkers may precede albuminuria, a marker of glomerular damage. In SCA, tubular injury is likely to precede glomerular injury due to recurrent ischemia within the renal medulla.

Evidence from experimental models and clinical studies supports a major role for TGF-β_1 in renal fibrosis [41, 42]. TGF-β_1 is the main modulator of the healing process after tissue injury. Under normal conditions its release ceases by feedback mechanisms when the healing process has been completed, but if TGF-β_1 release is not switched off, extracellular matrix components are accumulated and tissue fibrosis occurs. Upregulation of TGF-β_1 synthesis in the kidney is followed by the accumulation of collagen and scarring. It has been proposed that persistently high TGF-β_1 excretion correlates with morphological indices of chronicity. However, two recent cross-sectional studies in patients with SCD [19, 21] did not find a correlation between albuminuria and TGF-β_1. In our study, although there was an association between albuminuria and TGF-β_1 by the Pearson correlation coefficient, this biomarker could not be detected in 49 % of the samples, making the interpretation difficult. Sundaram et al. [19] also found very low or undetectable levels of TGF-$\beta_1$in a significant number of samples of patients with SCD. It is possible that TGF-β_1 may be a late marker so that at least half of our patients did not have detectable levels.

Another possible contributor to kidney disease is ET-1, a 21-amino acid peptide implicated in the development and progression of chronic kidney disease. It is produced by both the vasculature and the kidney. ET-1 is the most potent endogenous vasoconstrictor [43] and has a number of other major effects, including cell proliferation, inflammation and fibrosis [44]. ET-1 excretion increased twofold (3.86±0.95 vs. 1.65±0.68 pmol/h ; P<0.01) in SCD patients compared to healthy controls [24]. We were unable to demonstrate a significant difference between ET-1 levels between the patients with and without albuminuria; however, urine ET-1 levels were associated with severity of the anemia.

In addition to vaso-occlusion, chronic hemolysis may be the driving force behind several complications of SCA, including pulmonary hypertension and gallstones [45]. In children with SCA, significant associations have been found between proteinuria and the hemolysis markers of low hemoglobin and high LDH [46, 47]. Furthermore, iron itself may be deleterious to the kidney. Magnetic resonance imaging shows iron deposition within the kidney in individuals with SCD, and kidney iron deposition correlates with elevated LDH [48]. The presence of iron hydroxyl radicals can promote oxidative stress and tissue injury. Hemosiderinuria occurs with chronic intravascular hemolysis. Hemoglobin is released from red blood cells into the bloodstream in excess of the binding capacity of haptoglobin. The excess hemoglobin is filtered by the kidney and reabsorbed in the proximal convoluted tubule, where the iron portion is removed and stored in ferritin or hemosiderin. The proximal tubule cells eventually slough off with the hemosiderin; these cells are excreted into the urine. In our study, a strong association was found between albuminuria and LDH (P<0.001) and between albuminuria and hemosiderin (P<0.008).

Our study is limited by a relatively small sample size and cross-sectional design. Many of the patients with and without albuminuria had elevated levels of urine NAG and KIM-1 although those with albuminuria had higher levels. The relevance of the biomarkers remains unclear. Our results suggest that these markers may precede the development of albuminuria. Studies need to be performed longitudinally in order to determine whether the biomarker changes precede the presence of albuminuria and whether they may have prognostic significance for the progression of sickle cell nephropathy.

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