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ORIGINAL ARTICLE

Lactate dehydrogenase as a predictor of kidney involvement in patients with sickle cell anemia

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Abstract A retrospective chart review of 40 patients with sickle cell anemia (SCA) between the ages of 5–19 years who were seen within a 1-year period was performed to determine clinical and laboratory correlates for microalbuminuria and proteinuria. Age, sex, height, body mass index (BMI), serum creatinine [and estimated glomerular filtration rate (eGFR) by Schwartz and MDRD formulas], type of SCA, hemoglobin (Hb) level [total Hb and hemoglobin F percentage (HbF%)], lactate dehydrogenase (LDH) level, reticulocyte count, blood pressure, history of splenectomy, history of hydroxyurea use, and history of transfusions were correlated with microalbuminuria and proteinuria by univariate and multivariate regression analysis. The prevalence of microalbuminuria and proteinuria among these patients was 15 and 5%, respectively. Univariate analyses revealed a significant correlation between LDH level and microalbuminuria (Pearson r= 0.47, p=0.04) and between LDH level and proteinuria (Pearson r=0.48, p=0.035). Multivariate analysis revealed a significant correlation between microalbuminuria and LDH level (p=0.04) when controlled for age, sex, eGFR, Hb level, HbF%, type of SCA, BMI, history of transfusions, and reticulocyte count. In this pediatric SCA population, LDH was found to correlate with the presence of microalbuminuria and proteinuria. Further studies are needed to confirm LDH as an early marker for the risk of kidney involvement among SCA patients.

Keywords Lactate dehydrogenase · Microalbuminuria · Pediatric · Proteinuria · Sickle cell anemia

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Introduction

Renal failure due to sickle cell nephropathy is seen in 4–21% of adult sickle cell anemia (SCA) patients [1, 2]. It is one of the most serious complications of SCA and contributes to early mortality from the disease [3]. Duration of disease, severity of anemia, and genetic factors are believed to influence the risk of development of renal disease among patients with SCA [3–9].

SCA is associated with a wide spectrum of renal abnormalities. Early signs may include impaired urinary concentrating ability, defects in urinary acidification and potassium excretion, supranormal proximal tubular function manifested by increased phosphate reabsorption and increased creatinine secretion, glomerular hyperfiltration, and proteinuria [10]. As the disease progresses, nephrotic syndrome and end stage kidney disease can develop [10]. Several markers have been studied as early predictors of renal involvement, including estimated glomerular filtration rate (eGFR), microalbuminuria, and proteinuria. Patients with SCA have a prostaglandin-mediated supranormal GFR [11]. Glomerular hyperfiltration, regardless of etiology, is believed to eventually lead to glomerular sclerosis, proteinuria, and progressive renal failure [12]. GFR returns to normal levels with aging and frequently becomes subnor-

mal after age 40 years in patients with SCA [13]. Because of the increased creatinine secretion, creatinine clearance becomes an unreliable marker for the early detection of renal dysfunction among young patients with SCA.

Both microalbuminuria and proteinuria are common findings in adults with SCA [3, 14–16]. Several studies have revealed the prevalence of microalbuminuria to range from 19 to 46% and the prevalence of proteinuria to vary between 6 and 30% among children with SCA [4, 7, 17–19]. Microalbuminuria is one of the earliest markers of kidney damage and has been found to be directly related to age and inversely related to hemoglobin (Hb) levels among children with SCA [4]. Typically, microalbuminuria is not seen until children with SCA reach the age of 6–10 years [7, 17].

The aim of this study was to determine the prevalence of microalbuminuria and proteinuria in a pediatric SCA population at the Robert Wood Johnson Medical School/ Bristol-Myers Squibb Children's Hospital and to identify previously unstudied clinical and laboratory variables associated with microalbuminuria and proteinuria as early predictors of renal involvement among children with SCA.

Methods

Patients

After obtaining approval for this study from the UMDNJ Institutional Review Board, we performed a retrospective chart review on 40 patients with SCA [hemoglobinopathies: HbSS, HbSC, HbS thalassemia (HbSthal)] who were seen at the Sickle Cell Clinic at Bristol-Myers Squibb Children's Hospital between November 1, 2008, and November 1, 2009. Among the 142 patients screened, 40 were enrolled in the study based on the inclusion and exclusion criteria, reason for the visit, availability of medical records, and laboratory results. Any patient between the ages of 5–20 years with a diagnosis of SCA that was seen as an outpatient during the specified period was included in the study. Since the renal involvement correlates with the duration of SCA, patients younger than 5 years were excluded from the study. The data were collected from a pediatric clinic; therefore, there were no patients older than 19 years of age. During their regular visits, patients were screened for the presence of microalbuminuria and proteinuria with a first morning urine sample. Samples were not collected at sick visits. The medical records of these 40 patients were also reviewed for age, sex, weight, height, body mass index (BMI), type of SCA, Hb level [total Hb and hemoglobin F percentage (HbF%)], lactate dehydrogenase (LDH) level, reticulocyte count, blood pressure, history of splenectomy, history of hydroxyurea use, history of transfusions, serum creatinine, and eGFR (calculated by Schwartz [20] and MDRD [21] formulas based on patient's age). Hypertension was defined as systolic and/or diastolic blood pressure ≥95th percentile on three or more occasions based on previously developed standards for gender, age, and height [22].

Analysis

Exploratory data analyses were used to investigate the distribution of and relationship between variables. Means and standard deviations (SD) or median and range were used to summarize data where applicable. Two-sided tests of statistical significance, including the Pearson chi-squared coefficient and Fisher's exact test for categorical variables and Student's *t* test and Mann–Whitney test for continuous variables, were used. All variables were correlated with microalbuminuria and proteinuria by univariate and multivariate regression analysis. A p value <0.05 was considered to be significant. Statistical analysis was performed using Prism 5 by Graphpad Software (San Diego, CA).

Results

The final study population consisted of 40 SCA patients (male:female 20:20, age range 5–19 years, mean age 12± 4.5 years). Among these 40 SCA patients, 81.58% had HbSS, 13.16% had HbSC, and 5.26% had HbSthal disease. A total of 64.9% of all patients had a history of blood transfusions, and six patients (15%) were on chronic transfusion therapy. Twenty patients (50%) were on hydroxyurea therapy. Microalbuminuria (urine albumin/ creatinine >30 Mcg/mg) was present in six patients (15%). The prevalence of proteinuria (urine protein/creatinine >0.2 mg/mg) was 5% (2/40 patients). No child <12 years had microalbuminuria. The mean eGFR level was 175.8±37.5 mL/min/1.73 m^2, with a range of 125–277 mL/min/1.73 m^2. Two patients (5%) had hypertension and one (2.5%) had a BMI >95th percentile for age; these 3 patients did not have microalbuminuria or proteinuria and were therefore not excluded from the analyses. One patient was on an angiotensin converting enzyme inhibitor (ACEi) therapy and had microalbuminuria and proteinuria despite being on this treatment; therefore, he was included in analyses. Four of the six patients with microalbuminuria (66.7%) and one of the two patients (50%) with proteinuria were on hydroxyurea treatment. Only one of the patients with microalbuminuria and/or proteinuria was on chronic transfusion therapy.

Univariate analyses revealed a significant correlation between LDH level and microalbuminuria (Pearson r= 0.47, p=0.04) and between LDH level and proteinuria

(Pearson $r=0.48$, $p=0.035$). The repeat univariate analysis among patients with HbSS disease alone (33 patients) did not reveal a significant correlation with LDH levels.

Multivariate analysis revealed a significant correlation between microalbuminuria and LDH level ($p=0.04$) and an equivocal correlation between microalbuminuria and history of splenectomy ($p=0.05$) when controlled for age, sex, eGFR, Hb level, HbF%, type of SCA, BMI, history of transfusion, and reticulocyte count. When groups were categorized according to the presence of microalbuminuria, they were found to be significantly different for serum LDH level (Table 1), and the significant difference in the mean serum LDH level persisted when the analysis was repeated among HbSS patients alone ($p=0.041$). Further analyses also revealed a significant age difference between patients with and without proteinuria and a significant Hb level difference between patients with and without microalbuminuria (Table 1). The remaining categorical and continuous variables were not found to be significantly different between groups (Tables 1 and 2). The repeat analysis of categorical and continuous variables among HbSS patients alone revealed similar results (data not shown).

Discussion

Sickle cell nephropathy is an important cause of mortality among patients with SCA [3]. Renal injury is believed to start in childhood with progressive worsening of renal function into adult years [23]. ACEi and hydroxyurea treatments have been shown to be beneficial for the prevention and control of sickle cell nephropathy [7, 18, 24, 25]. Early detection of manifestations of renal involvement as well as early identification of risk factors for renal involvement can allow the clinician to offer a rational treatment for the SCA patient.

In an earlier study, we observed that children with SCA had elevated creatinine clearance and that this did not correlate with the prevalence of microalbuminuria or proteinuria [17]. Although our results on creatinine clearances need to be confirmed by more reliable studies of GFR, this finding may suggest a different mechanism for glomerular damage among patients with SCA. Our results indicate that LDH could be used as an early marker to identify the risk of renal involvement among patients with SCA. Serum LDH level is used as a surrogate measure of intravascular hemolysis. Hemolysis is a pathologic mechanism leading to cardiovascular, pulmonary, gastrointestinal, and renal manifestations in a variety of human diseases [26]. LDH elevation is also believed to be a marker of SCA patients with a syndrome of hemolysis associated nitric oxide (NO) resistance, endothelial dysfunction, and end-organ vasculopathy [27]. The degree of hemolysis varies among patients with SCA, and it has been proposed that chronic intravascular hyperhemolysis may represent a novel SCA sub-phenotype which may require new therapeutic strategies [28]. Hyperhemolysis has been found to be associated with premature mortality in adults with SCA [28]. The observed hemolysis-related organ damage is believed to arise from NO depletion via direct scavenging reactions with free plasma Hb and impaired NO generation due to enzymatic consumption of arginine by red blood cell arginase I [26–29]. Genetic factors are believed to influence the risk and type of renal involvement among SCA patients; however, whether the hyper-hemolysis sub-phenotype of SCA is a separate risk factor for renal involvement still needs to be explored.

There are several limitations to our study. Secondary to the cross-sectional design of this study, it is difficult to conclude a cause and effect relationship between the variables analyzed. Therefore, further studies are needed to confirm hyperhemolysis as an independent risk factor for renal involvement and microalbuminuria. Secondary to the previously identified

Table 1 Continuous variables

Variable	Microalbuminuria			Proteinuria		
	Microalbuminuria present	No microalbuminuria	Significance (p value)	Proteinuria present	No proteinuria	Significance (p value)
Age (years)	14.5 ± 3.9	11.6 ± 4.5	0.1609	18.5 ± 0.5	11.7 ± 4.4	0.0394
eGFR (mL/min/1.73 m^2)	176.2 ± 38.6	175.7 ± 37.2	0.9766	177.18 ± 48.8	175.7 ± 36.7	0.9591
Hemoglobin level (g/dL)	8.2 ± 1.1	9.5 ± 2.3	0.0478	8.8 ± 0.2	9.3 ± 2.2	0.6682
Reticulocyte count (%)	9.3 ± 4.5	7.0 ± 4.0	0.2291	9.4 ± 4.4	7.3 ± 4.2	0.5083
Hemoglobin F (%)	13.8 ± 13.2	11.1 ± 10.2	0.6146	15.5 ± 14.2	11.4 ± 10.6	0.6229
LDH (IU/L)	499.3 ± 118.1	366.5 ± 111.6	0.0062	525.5 ± 72.5	386.5 ± 125.8	0.0836

Values are given as the mean ± standard deviation

eGFR, estimated glomerular filtration rate; LDH, lactate dehydrogenase

Table 2 Summary of the comparison of categorical variables between patients with microalbuminuria and proteinuria

Variable	*n*	Microalbuminuria present (%)	Proteinuria present (%)	Significance (*p*)
Gender	Male: 20	20	5	NS
	Female: 20	10	5	
Type of sickle cell anemia (hemoglobinopathy)	HbSS: 33	12	6	NS
	HbSC: 5	0	0	
	HbSthal: 2	0	0	
History of splenectomy	Yes: 4	50	25	NS
	No: 36	11.1	2.7	
History of RBC transfusion	Yes: 26	16.5	8.1	NS
	No: 14	15.1	0	
HTN	Yes: 2	0	0	NS
	No: 38	15.8	5.2	
BMI >95th percentile	Yes: 1	0	0	NS
	No: 39	15.3	5.1	
Hydroxyurea treatment	Yes: 20	20	5	NS
	No: 20	10	5	

SCA, Sickle cell anemia; RBC, red blood cell; HTN, hypertension; BMI, body mass index; Hb, hemoglobin; NS, not significant

differences in severity of renal involvement in different SCA subtypes [9, 18], the analyses were repeated with HbSS patients alone to validate the results. Repeat univariate analysis of microalbuminuria and proteinuria among HbSS patients alone did not reveal a significant correlation with serum LDH levels; however, the mean serum LDH level was still significantly higher among HbSS patients with microalbuminuria than among HbSS patients without microalbuminuria. This could be due to the small sample size and needs to be validated in future larger studies that include higher numbers of patients of all SCA subtypes.

Similar to results reported in previous studies, we observed a significant difference in age between patients with and without proteinuria. However, there was no significant difference in age between patients with and without microalbuminuria. This result could be secondary to the small sample size. Previous studies reported no microalbuminuria among younger children [4, 7, 17], and in our study population we did not identify microalbuminuria in patients <12 years of age. Similar to previous studies, the patients with microalbuminuria had a significantly lower Hb level than patients without microalbuminuria; however, in our study group there was no significant difference in Hb levels between patients with and without proteinuria. This result should be interpreted with caution since the Hb levels used here reflected a single time point in a continuous disease process which may not reflect the overall severity of the disease.

Treatment with ACEi and/or hydroxyurea has been shown to be beneficial for the prevention of sickle cell nephropathy [7, 18, 24, 25]. In our study population, only one child was on ACEi therapy and had microalbuminuria; additionally, there was no significant difference with regards to prevalence of microalbuminuria and proteinuria with or without hydroxyurea treatment. This was likely secondary to the small sample size.

Unlike prior studies, our study identified a tendency for an association between history of splenectomy and microalbuminuria when controlled for other variables in multivariate analysis ($p=0.05$). Of the 40 patients in our study cohort, four had a history of splenectomy, and the mean age of these four patients was 12.7 years. Recurrent acute splenic sequestration crisis (ASSC) is the most common indication for splenectomy in SCA patients [30], and all four of these patients had splenectomy for recurrent ASSC. Overall, splenectomy is believed to improve patient outcomes by reducing the number of transfusions [30]; however, the need for splenectomy may suggest a more severe SCA course which could explain our findings. Of our four patients with a history of splenectomy, two had significant microalbuminuria, only one of whom also had significant proteinuria; the other two patients had normal urine microalbumin and protein levels. Secondary to the low number of patients, this association could also be due to chance and needs to be further explored in future studies.

In conclusion, the results of our study demonstrate a relationship between LDH level and microalbuminuria and proteinuria in a pediatric SCA population. Further studies are needed to confirm LDH level as an early marker for the risk of kidney involvement among SCA patients. This finding may enable prompt identification of patients who in turn may benefit from early intervention.

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