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RESEARCH ARTICLE

IRON ASSESSMENT IN SICKLE CELL DISEASE PEDIATRIC, KHARTOUN STATE, SUDAN 2017

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ABSTRACT

Background: vaso-occulusive event in pediatric sickle cell disease (SCD) may cause renal complication such as urinary concentrating defects, impaired urinary acidification, proteinuria, and hematuria. Chronic sickling promotes different mechanism of kidney injury. Iron accumulation due to hemolysis can cause various organ damage.

Objective: the study aimed to asses proteinuria, hematuria, urobilinogenuria and ferritin levels as indicators of sickle cell nephropathy (SCN)

Materials and Method: this is a cross sectional study, conducts in DrGaafarIbnauf Pediatric Tertiary Hospital in KHARTOUM, SUDAN from December 2016 to February 2017. It included a fifty pediatric in steady state of disease, with history of multiple blood transfusion, in administration of hydroxyuria and without chronic renal failure. The patients were screening for Kidney involvement features such as proteinuria, hematuria, and urobilinoge, in relation to anemia degree and hemolysis marker such as, hemoglobin and reticulocyte, and ferritin level

Results: prevalence of renal complication in our cross sectional study in pediatric sickle cell disease (mean age 3.8±1.1years) was found to be relatively high, predominately proteinuria (46%), hematuria (8%), and urobilinogenuria (10%). Urine concentration ability show an acidic urine, pH was 5.52±0.50 and low specific gravity value 1.00±0.01. Ferritin was show high level 451.01±392.17ng/ml.

Conclusion: In this studyin SCD. We observed proteinuria frequently detected, and less extent hematuria and urobilinogen, also high Iron concentration is a common feature.

INTRODUCTION

Vaso-occlusive phenomena and hemolysis are the clinical hallmarks of sickle cell disease. The polymerization of deoxy hemoglobin S results in distortion of the red blood cell (RBCs) into the classic sickle shape and a marked decrease in red cell deformability and is the primary cause of vaso-occlusive phenomena Subsequent changes in red cell membrane structure and function, disordered cell volume control, and increased adherence to vascular endothelium also play an important role.

Vaso-occlusion result in recurrent, painful episodes and a variety of serious organ system complications that can lead to lifelong disabilities and even death (Allen *et al*., 2012). Renal abnormalities are common complications of SCD. Improved care of patients with SCD has resulted in longer survival (Mario Abbud-Filho, 2013). Kidney abnormality in SCD start in childhood, with hematuria being the most common manifestation, along with renal papillary necrosis and tubular function abnormalities. Which are triggered by Vaso-occlusive phenomena.

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The long term consequence of this condition are sickle cell glomerulopathies, (albuminuria in 68% of SCD adults), with evolution to chronic kidney failure. In as many as 20% of homozygous patients (Guasch, 2006). The condition can be recognized early by paying attention to results of urinalysis, the development of hypertension, and falling hemoglobin concentration (Falk, 1992). Four different types of glomerulopathy have been described in SCD, FSGS, membranopro life rative glomerulopathies (MPGN), glomerulopathy specific to SCD, and thrombotic microangiopathy (TMP). Regardless of which type of glomerular damage is presented. All renal biopsies from SCD patients show hypertrophied glomeruli with sickled blood cells, and is also found in subject with SCD and no evidence of CKD. Haemosidrosis deposits in tubular cells are almost universal founded (Magine, 2010 and Bhathena *et al*., 1991). The development of renal impairment was heralded by evidence of early renal involvement, including ineffective erythropoiesis, hypertension, proteinuria (including the nephrotic syndrome) and hematuria (C.R.V, 1992). Progressive renal impairment may first noted because of worsening anemia, owing to impairment erythropoietin production. Repeat transfusion at this stage may result in iron over load (Morgan, 1982; Sherwood, 1985 and Allon, 1988).

Hematuria: This is the most common form of SCD. It can be microscopic or more commonly macroscopic and self-limiting. It is frequently unilateral, and is more often found in the left kidney, due to the longer left renal vein and its anatomical location, compressed between the aorta and the superior mesenteric artery this subjects this vessel to a greater venous pressure with relative hypoxia in the renal medulla that favors cell sickling. It can develop at any age, and has been primarily described in patients with sickle cell trait (HbAS) much more frequent than in the homozygous form (HbSS) (Weatherall, 2010; Modell, 2008; Odita, 1983 and Pandya, 1976). Hematuria is probably a consequence of cell sickling in the renal medulla combined with vascular obstruction and extravasation of erythrocyte. The medullary environments is by nature prone to producing sickle cell due to the partial pressure of O_2 at 35-45 mmHg, bellow the sickling threshold of 45 mmHg, along with high osmolarity that dehydrates erythrocytes and concentrates the HbS and the acidic pH that also increases the probability of sickling (Allon, 1990).

Proteinuria: The renal involvement responsible is a glomerulopathy whose initial marker is albuminuria. The prevalence increase with age, ranging between 21.3% and 28.8% in patients aged 3 to 40 years (Alvarvez, 2008 and Mckie, 2007). Proteinuria which can progress to the nephrotic syndrome, is the most common manifestation of glomerular injury in SCD patients. Moreover, as many as 40 percent of SCD-SS patients with nephrotic syndrome may go on to develop end-stage renal disease (ESRD). Therefore, patients with persistent proteinuria should have a urine collection obtained for the determination of 24hour protein excretion, and a nephrology consultation should be requested for consideration of other non-sickling causes of proteinuria and possible renal biopsy (Foucan, 1998; Powars *et al*., 1991 and De jong, 1980). Proteinuria is associated with higher levels of anemia, hemolysis, and reticulocytosis (Zayas, 1996).

Hemosiderin and Iron over load: The human body has no effective physiological mechanism for excreting excess iron. Therefore, in condition such as sickle cell disease (SCD), where transfusions are frequently indicated, exogenous iron can accumulate, circulates as non-transferrin bound iron (NTBI), enter tissues, from reactive oxygen species (ROS), and result in end organ damage. However, patients with SCD, compared with thalassemic patients, despite a similar transfusion load, may be relatively protected from iron mediated cardiac and endocrine gland toxicity (Walter, 2009). Iron hemostasis in human is maintained by the strict regulation of absorption based on body needs. 1 mg (10% of total dietary iron) is absorbed daily, predominately in duodenum, and an equal amount is lost through faces, urine and sweat (Dubach, 1955). In the absence of iron overload, some absorbed iron is stored in the enterocyte as ferritin and the rest is transported across the baso-lateral membrane by ferroportin, with the aid of Ferro-oxidase hephaestin. In the circulation, iron is bound to transferrin and transported to the liver and bone marrow. In the liver, transferrin receptors 1 and 2 mediate the endocytosis of iron, which is then restored ferritin and released by a ferroportin-mediated mechanism when bodily needs increase. In the erythroid precursors, transferrin bound iron is taken up by transferrin receptor 1 and utilized for erythropoiesis. During red cell senescence, iron is released into macrophages in the reticuloendothelial system (RES) and is stored as ferritin and hemosiderin. The only frequently demonstrated interstitial lesion is abundant hemosiderin granules in tubular epithelial cells (Fleming, 2005 and Magine). The gold standard for assessing live iron stores in the absence of cirrhosis is the hepatic iron content (HIC), determine by liver biopsy and quantitation with atomic absorption spectrophotometer. Noninvasive methods including blood tests (ferritin and iron saturation). Renal iron deposition has also been noted on magnetic resonance scans in patients with SCD but appears not related to liver iron concentration, a marker of total body iron load. Renal iron, however, does appears to be correlated with lactate dehydrogenase, a marker of hemolysis, but so far has not been shown too associated with renal dysfunction or degree of albuminuria. Ferritin has been shown to correlate with (HIC) in TM, but the correlation in SCD is less clear (Angelucci,, 2000; Schein and Telfer, 2000).

MATERIALS AND METHODS

Study area and population: The study population consist of 50 randomized multiple transfused pediatric patients with SCD in steady state of disease, they also under hydroxyuria treatment who administrated to DrGaafarIbnauf Pediatric Tertiary Hospital, in KHARTOUM, SUDAN. The study was conducted from December 2016 to February 2017. The prospective cross- sectional study was approved by AL_Neelain University committees. The laboratory investigation were discus with patients parents, and written consents were taken, the mean age of population is 3.8±1.1years.

Sample collection: A total of 5 ml blood was drown from each subject, 2.5 ml in EDTA container for CBC and Reticulocytecountand 2.5 ml in plain container for ferritin assay ; serum was separated in 0.5 ml eppendorf tube and stored in -20oC until analysis, CBC was done usingsysmexKX-21N, Reticulocyte countwas doneby newmethylene blue stain (Mitchell lewis, 2006 and Roche, 2017), ferritin was measured using ROCHE ELICSYS 2010 (Roche, 2017). Also Urine sample was collected for determine Blood, protein, and urobilinogen a mission® expert urinalysis reagent strip was used, also color pattern, pH and specific gravity was observed.

Statistical analysis: experimental data are presented in arithmetic mean, standard deviation and standard error were calculated by SPSS version (20),a 1 tailed t-test was calculated and P-value of < 0.05 was considered as statistically significant value. R-value was used to measure relationship between variables, $-1 \geq 0 \leq 1$ is a significant

Table 1. Show Hematological and urine parameters

Parameters	Mean ±SD	Mean (R.V) 2-6 years	*P-value*
RBCS $\times 10^{12}$/l	2.68±0.59	4.6±06	0.000
HBg/l	77.9±14.5	125±15	0.000
PCV l/l	0.24±0.05	0.37±0.03	0.000
reticulocyte count $\times 10^{9}$/l	216.0121±39.12	65 (30-100)	0.000
Ferritin ng/ml	451.01±392.17	75 (10-140)	0.000
Urine specific gravity	1.00±0.01	1.015 (1.0-1.03)	0.044
Urine Ph	5.52±0.50	6 (4.5-8)	0.000

RESULTS

In this cross-sectional study there were afifty patients under investigation, with female predominately (62%), male (38%), age range 3.8±1.1years. Table (1) shows the mean and the

relation between a case and reference values of RBCS count, HB, HCT, Reticulocytecount, Ferritin level, and urine s`pecific gravity and pH. Hematological parameters were significantly altered from normal range of healthy pediatric. The mean RBCs count among case was 2.68±0.59×10^{12}/l, and among reference range 4.6±06×10^{12}/l (p.value 0.000), hemoglobin 77.9±14.5g/l in compare with reference125±15g/l (p-value 0.000), packed cell volume0.24±0.05l/land 0.37±0.03 l/l among reference (p-value 0.000), also absluotereticulocyte count was 216.0121±39.12×10^{9}/land65 (30-100) ×10^{9}/lin reference (p-value 0.000), ferritin level was451.01±392.17ng/ ml in compare with 75 (10-140) ng/ml for reference (p-value 0.000).

was calculated, no significant association was found other than with ferritin (p-value 0.003). Urobilinogen as hemolytic marker was found in in 10% (5 patients from 50). No significant association was found. Table (5) shows the most important finding that revealed the relationship between study variables using p-value and correlation coefficient, There is a nearly perfect positive relationship at 0.01 level between hemoglobin and hematocrit (p-value 0.000, R-value 0.965), a strong positive at same level between RBCs count and hemoglobin (p-value 0.000, R-value 0.717), RBCs count and hematocrit (p-value 0.000, R-value 0.704). Also there is a moderate negative at same level between reticulocyte and RBCs count (p-value 0.000, R-value 0.-0.572), and weak

Table 2. Proteinuriaassociation with hematological state

Variables	Proteinuria	N (%)	Mean±SD	*P-value*
RBCS×10^{12}/l	Nil	27 (54%)	2.61±0.54	0.341
	+	23 (46%)	2.77±0.65	
HB g/l	Nil	27 (54%)	74.3±10.0	0.054
	+	23 (46%)	82.2±17.7	
HCTl/l	Nil	27 (54%)	0.23±0.03	0.041
	+	23 (46%)	0.26±0.06	
Reticulocyte count×10^{9}/l	Nil	27 (54%)	207.73±31.78	0.473
	+	23 (46%)	224.07±45.51	
Ferritinng/ml	Nil	27 (54%)	413.133±379.27	0.003
	+	23 (46%)	427.08±253.1309	

Table 3. Urobilinogen association with hematological state

Variables	Urobilinogen	N(%)	Mean±SD	*P-value*
RBCS×10^{12}/l	Nil	45(90%)	2.71±0.59	0.270
	++	5(10%)	2.41±0.51	
HB g/l	Nil	45(90%)	78.9±14.7	0.067
	++	5(10%)	69.0±8.9	
HCT l/l	Nil	45(90%)	0.25±0.05	0.037
	++	5(10%)	0.22±0.02	
Reticulocyte×10^{9}/l	Nil	45(90%)	218.05±39.96	0.619
	++	5(10%)	179.65±26.76	
Ferritinng/ml	Nil	45(90%)	404.82±331.417	0.210
	++	5(10%)	547.6±261.513	

Table 4. Hematuriaassociation with hematological state

Variables	Hematuria	N(%)	Mean±SD	*P-value*
RBCS×10^{12}/l	Nil	46(92%)	2.66±0.61	0.086
	+	4(8%)	2.94±0.22	
HB g/l	Nil	46(92%)	78.4±14.9	0.110
	+	4(8%)	72.0±5.6	
HCT l/l	Nil	46(92%)	0.24±0.05	0.100
	+	4(8%)	0.22±0.02	
Reticulocyte count×10^{9}/l	Nil	46(92%)	213.79±39.36	0.394
	+	4(8%)	241.53±22.13	
Ferritinng/ml	Nil	46(92%)	408.92±326.728	0.001
	+	4(8%)	511.6±335.577	

There is a significant association showed in all variables and reference value. Urine parameters such as pH, and specific gravity are not in normal range. Urine pH were 5.52±0.50 and in a reference 6 (4.5-8), (p-value 0.000) And value of specific gravity was 1.00±0.01, were they 1.015 (1.0-1.03) in reference, (p-value 0.044) no significant alternation in patients. In majority of subjects' urine biochemical and physical parameters' are found to be altered. This may due to the side effect of chelating drug, deposition of Iron in renal tissue, renal infraction due to high platelets aggregation, low serum level of protein and anti-thrombin III (Eldor, 1993). Table (2) shows Proteinuria was found in 46% of subjects (23 out off 50), Association between proteinuria and hematological parameters

negative with hemoglobin concentration (p-value 0.007, R-value -0.375). And at level of 0.05 there is a weak negative relationship between RBCs count and ferritin (p-value 0.015, R-value -0.343), and between hematocrit and reticulocyte count (p-value 0.019, R-value -0.331).

DISCUSSION

A number of studied have shown that proteinuria is the earliest sign of renal disease in SCD patients (Ataga *et al*., 2000; Gausch, 1996; Schmitt, 1998; McBurney, 2002). Our finding that proteinuria was common in pediatrics SCD in keeping with that of other study.

Table 5. Relationship between study variables with each others

Variables		RBCS	HB	HCT	Retic count	ferrittin	S.G	pH
RBCS	*R-value*		0.716**	0.704**	-0.572**	-0.343*	0.178	0.043
	P-value		0.000	0.000	0.000	0.015	0.215	0.767
HB	*R-value*	0.716**		0.965**	-0.375**	-0.126	0.198	0.064
	P-value	0.000		0.000	0.007	0.385	0.167	0.659
HCT	*R-value*	0.704**	0.965**		-0.331*	-0.083	0.185	0.043
	P-value	0.000	0.000		0.019	0.566	0.198	0.767
Reticulocyte	*R-value*	-0.572**	-0.375**	-0.331*		0.279	-0.206	-0.103
	P-value	0.000	0.007	0.019		0.050	0.152	0.478
Ferritin	*R-value*	-0.343*	-0.126	-0.083	0.279		-0.141	-0.087
	P-value	0.015	0.385	0.566	0.050		0.329	0.546
Urine S.G	*R-value*	0.178	0.198	0.185	-0.206	-0.141		0.106
	P-value	0.215	0.167	0.198	0.152	0.329		0.462
Urine pH	*R-value*	0.043	0.064	0.043	-0.103	-0.087	0.106	
	P-value	0.767	0.659	0.767	0.478	0.546	0.462	

The prevalence was observed to increase 46%. Our prevalence was high than the 24% found by McBurney *et al* 2002 (McBurney, 2002), 14.3% found by Datta *et al* 2002[36], 9.4% found by Osei *et al* 2008 (CT Osei-Yeboah, 2011), and 3.2% by Stallworth JR *et al*l 2011 (Stallworth, 2011), Proteinuria was correlated with only elevated ferritin. Hematuria it was found to be 10% in comparison with 6.3% found by Stallworth JR *et al*l 2011. A mean urine pH in our study found to be 5.52±0.50 (acidic urine) which is lower than normal pH level 7.4 (7.35-7.45) and lower than 6.57+0.523 found by Andhale RB *et al* 2013 (Andhale, 2013), and specific gravity of 1.00±0.01, no significant change was seen in compare with normal range 1.015 (1.0-1.03) and 1.015 (1.0-1.03) found by Andhale RB *et al* 2013 (Andhale, 2013). Ferritin was found to be higher than normal range75 (10-140), a mean of 451.01±392.17ng/ml were found. A study by M A Hussain *et al* 1978[40] ferritin was found to be 367 ng/ml.

Biomarker of hemolysis

Hemoglobin concentration: Low hemoglobin is the most useful biomarker in SCD. A mean concentration of hemoglobin was found to be 77.9±14.5 g/dl which is lower than the reference range.

Reticulocyte count: Reticulocyte count correlates well with RBCs life span and is a routine procedure for assessment of bone marrow response. A mean of 216.0121±39.12×10^9/lwas found, and it is higher than normal range

Urobilinogen as indicator of hemolysis in urine was found to be 8%: All hematological parameters were found to be significantly lower than normal range of matched age health pediatrics, except in Red cells count no significant alteration were found.

Conclusion

In this study, We observed proteinuria frequently detected, and less extent hematuria and urobilinogen, also high Iron concentration is a common feature.

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