Renal manifestations of sickle cell disease in children

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ABSTRACT

Background: Sickle cell disease prevalence is more in central part of India. This study tries to find extent of renal involvement, risk factors and screening tests in sickle cell disease.

Methods: Study was Cross sectional observational study. Demographic and clinical findings were recorded. Renal function tests like serum creatinine, blood urea nitrogen, eGFR were studied. Presence of microalbuminuria was checked. Ultrasonography abdomen was done to see the texture and corticomedullary differentiation of kidney.

Results: Total 143 patients were studied. Out of which, 117 homozygous (SS type), 26 heterozygous (AS type). Majority belonged to school going age group (i.e. 5 to 10 year age group) with male preponderance in homozygous state. Microalbuminuria was more common in crisis state (61.84% in SS pattern and 83.33% in AS pattern) as compared to steady state in both SS and AS pattern patients. Dipstic method detected more proteinuria than heat coagulation suggesting dipstic method superior to heat coagulation test. Low eGFR was common in patients with SS pattern as compared to patients with AS pattern and was significantly associated with microalbuminuria in both steady state and crisis state. Abnormal USG was seen 3.44% in patients with SS pattern. Renal involvement started below 5 years of age and then increases with age, homozygous childrens in crisis state are affected more.

Conclusions: Renal involvement is common in sickle cell anemia i.e it is seen in 50% of the patients. Increasing age, male sex and homozygous state were risk factors. Simple test like dipstic method and heat coagulation test can be used as screening test to detect microalbuminuria, with dipstic method being superior. Presence of microalbuminuria can detect early renal involvement in sickle cell disease. This will help in early diagnosis and management of such patients which avoids further renal complications and thus prevents mortality and morbidity.

Keywords: Blood urea nitrogen, eGFR, Microalbuminuria, Sickle cell anemia, Seum creatinine

INTRODUCTION

Sickle cell anaemia (SCA; homozygous sickle haemoglobin [HbS], i.e. HbSS) occurs when thymine is substituted for adenine in the 6th codon of the beta globin gene, resulting in the production of valine (a hydrophobic amino acid) instead of glutamic acid, which is hydrophilic. In sickle cell anaemia, HbSS is commonly as high as 90%of total haemoglobin; whereas in sickle cell disease, HbSS more than 50% of total haemoglobin. The polymerization of Hb S within red blood cells (RBCs) (sickling) on deoxygenation underlies all the pathophysiology of SCD. As Hb S-containing RBCs traverse the circulation undergoing cycles of oxygenation and deoxygenation, rigid polymers of Hb S repeatedly form and damage the RBC membrane, drastically shortening the RBC life span. RBCs also become dehydrated, relatively inflexible, and abnormally adhesive. Consequently, they are prone to adhere to the endothelium of blood vessels, in concert with leukocytes.
and platelets, impeding the flow of blood. This microvascular obstruction, called vaso-occlusion, leads to ischemia, infarction, and ischemia-reperfusion injury of multiple organs including kidneys.

Increased renal plasma flow in SCD is accompanied by increased renal blood flow and GFR. Increased GFR subsides to a normal GFR with either ageing or development of CKD, and to a subnormal GFR as CKD progresses. Hyperfiltration can occur as early as in infancy in patients with SCD. Filtration fraction is reduced in patients with SCD, and this observation might reflect the selective loss of juxtamedullary nephrons, which exhibit a higher filtration fraction than do cortical nephrons. In young adults who have SCD and exhibit renal hyperperfusion and hyperfiltration, functional measurements and mathematical modelling demonstrate that hyperfiltration is principally driven by increased glomerular plasma flow rate and glomerular ultrafiltration coefficient (Kf); elevations in transcapillary hydraulic pressure gradient values are marginal.

The basis for renal hyperperfusion remains unresolved. Hyperperfusion likely reflects the activity of vasorelaxant species, especially because renal vascular resistance (RVR) is reduced. Such vasorelaxant species include prostaglandins, the production of which increases in the kidney of patients with SCD, possibly because of medullary ischaemia. Increased production of kallikrein might also underlie renal hyperperfusion. A possible basis for renal hyperperfusion involves the heme-oxygenase–carbon monoxide (HO-CO) system. In contrast to whole-kidney blood flow, medullary blood flow is regarded as being markedly reduced in SCD.

Apart from that hyperfiltration hyperperfusion injury also causes progressive glomerular damage leading to microalbuminuria, which might act as early marker for glomerular disease. Microalbuminuria precedes gross persistent proteinuria, which is subsequently followed by renal failure in sickle cell disease specially with increasing age 3-7. Renal involvement is also seen in homozygous state.

In this study, the spectrum of renal involvement in sickle cell disease children is assessed by evaluation of Renal functions (Serum urea, creatinine, estimated GFR), and proteinuria. It also predicts risk factors which facilitate the progression of renal disease and the markers for early detection of renal involvement like microalbuminuria.

**METHODS**

Present study was cross sectional observational study conducted in Pediatric wards at Shri Vasantrao Naik Government medical college and hospital, Yavatmal in Vidharbha part of Maharashtra for a period of one year from 1st march 2018 to 28th February 2019.

**Inclusion criteria**

All diagnosed cases of sickle cell disease (i.e. homozygous SS and heterozygous AS) from 6 months to 12 years admitted in Paediatric ward.

**Exclusion criteria**

- Patients with other haemolytic disease like thalassemia, hereditary spheroctysis, G6PD deficiency etc.
- Patients who refused to give consent.

Homozygous patients (SS type) in Crisis as well as in Steady state were included. Steady state patients who were free of crisis for at least 15 days only were enrolled. These patients were coming for regular follow up in Pediatric outpatient department.

A detailed clinical history of each patient at time of presentation was obtained and entered in a predesigned proforma. Data regarding previous medical history was obtained from previous records available with the patient. History taking was followed by complete clinical examination. Each informant was explained about the nature and purpose of this study and their written informed consent was obtained. All patients with homozygous sickle cell anemia and heterozygous sickle cell trait were analyzed using investigations like- CBC, sickness, serum urea and creatinine, urine routine microscopy, urine for proteins and USG abdomen for any pathology in kidney texture and corticomedullary differentiation were done.

**Laboratory analysis**

The following laboratory information were collected and analysed.

- All the blood sample collected was labeled and sent to pathology and biochemistry laboratory. The sample for Complete blood count (CBC) were collected in EDTA (ethelenediaminetetraacetic acid).CBC was processed by Benesphaera 3 part haematology analyzer
- Urine routine microscopy (10 ml freshly voided midstream urine sample collected in universal sterile bottle) was done and proteins in urine were analyzed by urine chemistry analyzer (SD Urometer120) using urostitx method.
- RFT (Renal function test)- Serum urea estimated using GLDH kit and serum creatinine by using alkaline picrate kit.
- Proteinuria was determined by dipstic method and heat coagulation method. In dipstic method, the strips were impregnated with reagent called tetrabromophenol blue, buffered to an acidic pH of 3, which reacts with albumin in the urine in 30-60 sec forming chromogen which yields colour change (pale green-green-blue). In heat coagulation method, a test
tube containing about 10 ml of urine is heated in its upper part until it boils. The precipitate which does not disappear after addition of three drops of concentrated acetic acid suggests proteinuria.

- Ultrasonography abdomen was done to see the texture and corticomedullary differentiation of kidney.

All patients were grouped in to crisis state and steady state.

Renal dysfunction was defined as the presence of at least one of the following criteria

- The diagnosis of high eGFR (hyperfiltration) or low eGFR using Schwartz formula. eGFR=K(height in centimeters)/serum creatinine. K is constant in first year of life, children>1 year, k=0.55). Hyperfiltration was defined as a GFR greater than 140 ml/min/1.73m² and low GFR (Chronic renal failure) was described as GFR less than 80 ml/min/1.73m².

- Patients were considered to have renal insufficiency if total serum Creatinine concentrations and blood urea nitrogen (BUN) were greater than upper limits of normal for age.

- Presence of microalbuminuria was considered when albumin excretion was in the range of 30-300 mg/dl from dipstick and heat coagulation method.8,9 The dipstick was reported as negative, trace (10-29 mg/dL), 1+ (30-100 mg/dL), 2+ (100-300 mg/dL), 3+ (300-1000 mg/dL), and 4+ (>1000 mg/dL). i.e. Microalbuminuria is said to be present if urine protein is between +1and +2.

- Heat coagulation was reported as Grade 0-Urine clear, Grade 1+ =Barely hazy, Grade 1+= Hazzy, Grade2+ =Cloudy, Grade3+ = Thick white, Grade 4+= =White precipitates at bottom.

- Medico renal disease (MRD) in USG kidney suggest changes in the echo architecture of the renal parenchyma like loss of corticomedullary differentiation.10

Definitions of important terminologies in present study

- Homozygous (SS) sickle cell disease: People who inherit two genes for hemoglobin (S), one from each parent with percentage of HbS as high as 90% of total hemoglobin.11

- Heterozygous (AS) state: people who inherit one sickle cell gene (S) from one parent and one normal gene (A) and presence of around 40% of HbS on electrophoresis.11

- Crisis state is defined as presence of either vasooclusive or aplastic or splenic sequestration or haemolytic crisis and steady state patients are those who were free of crisis for 15 days.12-14

- Vasooclusive crisis - Acute sickle cell pain is characterized by unremitting discomfort that occurs in any part of body but most often occurs in chest, abdomen and extremities.12

- Splenic sequestration - Abrupt painful splenic enlargement, often associated with acute drop in hemoglobin often 2gm/dl or more and thrombocytopenia.13

- Aplastic crisis- severe anemia characterized by haemoglobin below baseline and reticulocytopenia was called aplastic crisis.13

- Hematuria is defined as the presence of at least 5 red blood cells (RBCs) per microliter of urine.15

Data management and statistical analysis

Statistical analysis was performed using the statistics software SPSS for windows (17.0 SPSS, Chicago). The analysis of Student’s t-test was used for comparisons of means. Categorical variables were compared using Chi square test and Fischer’s exact test. A p value, <0.05 was considered significant.

RESULTS

By Dip stick method 38 (32.75%) SS patients had proteinuria 1+, followed by 2+ (7.75%) and 3+ (2.58%), suggesting that 50% patients of SS pattern had renal involvement in the form of proteinuria ranging from trace to 3+. In AS pattern, 23.07% had proteinuria 1+, followed by 2+ (3.84%). Renal involvement in the form of proteinuria (trace to 2+) was present in 38.4% AS patients.

<table>
<thead>
<tr>
<th>Dip stick</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>58</td>
<td>9</td>
</tr>
<tr>
<td>AS</td>
<td>58</td>
<td>17</td>
</tr>
</tbody>
</table>

Figure 1: Distribution of proteinuria by dipstick and heat coagulation in AS and SS.

By Heat coagulation test, 9 (7.55%) SS patients were having trace proteinuria, 10 (8.62%) were having grade 1 proteinuria and 3 (2.58%) were having grade 2. Although nearly 81% SS patients did not show proteinuria by above method, which was almost 50% by dipstic method. In AS pattern, 4 (15.3%) patients were having trace proteinuria, 1 (3.84%) had grade 1 proteinuria (Figure 1).

Low eGFR was seen in 33 (28.44%) followed by 2 (1.72%) patients with high eGFR in SS pattern. This suggests that 31.1% patients had abnormal eGFR in SS patients. In AS patients low eGFR was seen in 2 (7.69%)
followed by 1 (3.84%) patient with high eGFR. This suggests that 11.5% patients had abnormal eGFR in AS patients. Thus low eGFR was significant finding in both the groups. Abnormal blood urea nitrogen was seen in 32 (27.58%) patients in SS pattern and 3 (11.53%) in AS pattern.

Abnormal creatinine was seen in 36 (31.03%) SS patients and 4 (15.38%) AS patients. Abnormal USG finding was seen in 4 (3.44%) SS patients. Whereas no AS patients had abnormal USG findings. Hypertension was seen in 21 (18.10%) SS patients and only 1 (3.84%) AS patient.

<table>
<thead>
<tr>
<th>Microalbuminuria</th>
<th>Crisis</th>
<th>Steady</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low eGFR</td>
<td>High/Normal eGFR</td>
</tr>
<tr>
<td>Present</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Absent</td>
<td>06</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>32</td>
</tr>
<tr>
<td>p value</td>
<td>0.006</td>
<td>0.01</td>
</tr>
</tbody>
</table>

This association between microalbuminuria and low eGFR is not seen in AS patients

<table>
<thead>
<tr>
<th>Microalbuminuria</th>
<th>Crisis</th>
<th>Steady</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Low eGFR</td>
<td>High/Normal eGFR</td>
</tr>
<tr>
<td>Present</td>
<td>03</td>
<td>02</td>
</tr>
<tr>
<td>Absent</td>
<td>00</td>
<td>01</td>
</tr>
<tr>
<td>Total</td>
<td>03</td>
<td>03</td>
</tr>
<tr>
<td>p value</td>
<td>0.28</td>
<td>0.06</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SS (%)</th>
<th>AS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria by dipstick method</td>
<td>58 (50%)</td>
<td>09 (34.61)</td>
</tr>
<tr>
<td>Raised serum creatinine</td>
<td>36 (31%)</td>
<td>04 (15.38)</td>
</tr>
<tr>
<td>Abnormal eGFR</td>
<td>35 (30.17%)</td>
<td>03 (11.53)</td>
</tr>
<tr>
<td>Abnormal BUN</td>
<td>32 (27.58%)</td>
<td>03 (11.53)</td>
</tr>
<tr>
<td>Proteinuria by heat coagulation</td>
<td>22 (18.96%)</td>
<td>05 (19.23)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>21 (18.10%)</td>
<td>01 (3.84)</td>
</tr>
<tr>
<td>Hematuria</td>
<td>10 (8.6%)</td>
<td>00</td>
</tr>
<tr>
<td>Abnormal USG kidney</td>
<td>04 (3.44%)</td>
<td>00</td>
</tr>
</tbody>
</table>

In SS patients, microalbuminuria was more common in crisis state (61.84%) than in steady state (27.5%) (Table 1). In AS patients also microalbuminuria was more in crisis state (83.33%) than in steady state. This difference was statistically significant (p value < 0.05%) (Table 2). In both crisis and steady state, microalbuminuria was associated with low eGFR, the difference was statistically significant (p value < 0.05%); in SS patients (Table 1).

**DISCUSSION**

The study group comprised of 142 patients of which 116 were sickle cell disease (SS pattern) and 26 were sickle cell trait (AS pattern). There were 6000 admissions in Pediatric ward during study period of which 370 patients were sickle cell disease (SS and AS pattern) thus the prevalence of study is 6.1% which was nearly equal to other study carried out by Kamble M et al.16

In this study, in both SS and AS group, maximum number of patients were between 5 to 10 years i.e. school going age group with male children affected more than female. On an average, 74% patients hospitalized were between 5-12 yrs. Male patients outnumbered females in SS pattern as observed in other studies. Total number of patients of SS pattern were 116 out of which 64 (55.17%) were males and 52 (44.82%) were females. Total number patients with AS pattern were 26 out of which 13 (50%) were males and 13 (50%) were females. Male to female ratio was 1.23:1 in SS pattern and 1:1 in AS pattern. The reason for male predominance may be due to good care for males with more hospital visits for them, male predominant society and neglect to female child.

In SS patients, total 50% patients were having proteinuria by dipstick method with gradings ranging from trace (6.89%), 1+ (32.75%), 2+ (7.75%), 3+ (2.58%). By heat coagulation method, 18.97% patients had proteinuria ranging from trace (7.75%), grade 1 (8.62%), grade 2 (2.58%). This suggest that microalbuminuria (1+ and 2+) was more common. Prevalence of microalbuminuria was found to be 40%. Study carried out by Yee M et al, showed that 27% patients had microalbuminuria which is lower than present study.17 Variation in results may be because of method used for the detection of proteinuria i.e. by dipstick and heat coagulation method which are not gold standard for the detection of proteinuria.18
In AS patients, total 34.61% patients were having proteinuria with dip stick method with different gradings ranging from trace (11.53%), 1+ (23.07%) followed by 2+ (3.84%). By heat coagulation method, only 19.23% patients were having proteinuria with different grades ranging from trace (15.3%), grade 1(3.84%). Prevalence of microalbuminuria (1+ and 2+) was 26%. Study carried out by Yee M et al. showed that 27% patients had microalbuminuria almost similar to the study in AS patients.\textsuperscript{17}

In this study microalbuminuria was significantly associated with crisis state in both group but highly significant in sickle cell disease (SS) group (p value=0.002) as observed in other studies like study carried out by Lakhkar BB.\textsuperscript{14}

Hematuria was present in 8.64% patients in present study. Cause of the hematuria is probably related to the pathologic events in the inner medulla and renal papillae of patients with sickle cell disease. Sickling of papillae in sickle cell nephropathy are due to occlusion of erythrocytes in the vasa recta results in increased blood viscosity, microthrombi formation, and ischemic necrosis, which, in turn, causes structural changes leading to hematuria.\textsuperscript{19}

Abnormal blood urea nitrogen was seen in 27.58% in SS patients and 11.53% in AS patients. Abnormal creatinine was seen in 31.03% in SS and 15.38% in AS patients. Aloni MN et al, creatinine tends to be more abnormal in children with Hb-SS than in Hb-AA subjects.\textsuperscript{20}

The crisis state was more common in SS group (65.51%) than AS group (23.07%) and this difference was statistically significant (p=0.004). In this study, age and crisis state are not associated with each other in both SS and AS pattern patients (p=0.47 and p=0.17).

In SS patients low eGFR was seen in 33 (28.44%) followed by 2 (1.72%) patients with high eGFR and in AS group low eGFR seen in 02 (07.69%) followed by 1 (3.84%) patients with high eGFR.

In this study microalbuminuria was significantly associated with crisis state in both group but highly significant in sickle cell disease (SS) group (p=0.002). It was observed that detection of proteinuria by dip stick was more superior than by heat coagulation test. Also dipstick test is more convenient and easy to perform.\textsuperscript{18}

In this study, in case of SS group out of 58 patients of crisis state, 47 patients (81.03%) were having microalbuminuria of which 23 (39.56%) patients had low eGFR. Out of 58 patients in steady state only 11 (18.96) patients were having microalbuminuria of which only 3 (5.17%) patients had low eGFR. This association between microalbuminuria and eGFR in both steady and crisis state was statistically significant (p= 0.006 and p= 0.01).

In case of AS group out of 06 patients in crisis state, 05 patients had microalbuminuria (83%) of which 03 (50%) patients had low eGFR. Out of 20 steady state, only 04 (20%) patients were having microalbuminuria of which only 1 (5%) patient were having low eGFR. This association between microalbuminuria and eGFR in steady and crisis state was not statistically significant. (p= 0.28 and p=0.06).

In present study the sensitivity, specificity and positive and negative predictive values of the dipstick test for detection of protein were found to be 81.0%, 93.1%, 21.2% and 98.6%.

In this study hypertension was seen in 18.10% of SS patients and 3.84% of AS patients. Study by Lakhkar BB showed that 11% patients had hypertension in crisis state.\textsuperscript{19}

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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