Renal involvement and its detection in sickle cell disease children

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ABSTRACT

Background: Sickle cell disease is common in this part of India. Study tries to find extent of renal involvement, risk factors and screening tests in sickle cell disease.

Methods: Convenient Prospective, cross sectional, observational study among known sickle cell anemia (homo and heterozygous) children and Fifty controls. Demographic and clinical findings were recorded. Renal function tests including serum sodium and potassium, eGFR were studied. Presence of microalbuminuria was checked and renal Doppler study was performed.

Results: Total 110 homozygous (SS type), 55 heterozygous (AS type) and 50 normal children were recruited. Majority belonged to low socioeconomic state with male preponderance. More patients in crisis had high Urea (23%) and Creatinine (43%) than in steady state (high urea 3.38%, high creatinine 15%). In AS type children also high urea (3.6%) and creatinine (11%) was found. A subgroup in SS and AS type had lower than normal urea and creatinine. Sodium and potassium abnormality also was found in both groups. eGFR was high below 5 years and then came down. All parameters worsened with age. Renal Doppler was better indicator of renal damage than microalbuminuria.

Conclusions: Renal involvement is common in sickle cell anemia. Increasing age, male sex and homozygous state were risk factors. Renal Doppler and micro-albuminuria are good screening tests. Renal Doppler was marginally superior.

Keywords: Sickle cell anemia, Kidney functions, Microalbuminuria, Renal Doppler

INTRODUCTION

Sickle-cell anaemia is caused by a point mutation in the β-globin chain of haemoglobin, leading to replacement of glutamine by valine at 6th position in the molecule.

The loss of elasticity of red blood cell is the main phenomenon in pathophysiology of disease. Elasticity of normal cell, allows it to change its shape so that it passes through capillaries. In sickle-cell disease, hypoxia causes sickling and repeated episodes, harm the cell membrane permanently and decrease elasticity. These cells also fail to return to normal shape on restoration of oxygen tension. The rigid cells are unable to pass through small capillaries, leading to vaso-occlusion. The crises in sickle cell disease affect different organs including kidneys.

The kidney is an organ of considerable impact on the clinical course of sickle cell patients. The renal manifestations of Sickle Cell Disease (SCD) have been described to range from various functional abnormalities to gross anatomic alterations. The environment of renal medulla which is hypoxic and hypertonic is known to predispose to sickling, which significantly decreases renal medullary blood flow through vaso-occlusion.1 This is the main mechanism of renal involvement.

Apart from that hyperfiltration hyperperfusion injury also causes progressive glomerular damage2 leading to
microalbuminuria, which might act as early marker for glomerular disease. It has been observed by other authors that a prolonged period of microalbuminuria precedes gross persistent proteinuria, which is subsequently followed by renal failure in sickle cell anemia specially with age increase. \(^3\) Renal involvement is also remarkable in heterozygous state.

Present study is carried out to know the spectrum of renal involvement in sickle cell disease children by comparing Renal functions (Serum urea, creatinine, estimated GFR), and serum electrolytes (Serum Na, serum K) with normal children. An effort is also made to study risk factors which facilitate the progression and to compare the markers for early detection like microalbuminuria and renal Doppler.

**METHODS**

This cross sectional observational study was conducted at the department of pediatrics, Acharya Vinoba Bhave rural hospital, Jawaharlal Nehru medical college, Sawangi (Meghe), Wardha for a period of two years from 1\(^{st}\) August 2012 to 31\(^{st}\) July 2014. Following were patient selection criteria.

**Inclusion criteria**

1. Children between 6 months to 15 years having homozygous or heterozygous sickle cell anemia already diagnosed by sickling test and Hb electrophoresis.

2. Normal children (AA type) in whom sickling test and Hb electrophoresis was done and sickle cell anemia was excluded acted as controls.

**Exclusion criteria**

1. All homozygous (SS) and heterozygous (AS) children with congenital urogenital anomalies.

2. Children with prior known conditions causing proteinuria, like hypertension, diabetes, HIV, HCV, renal and cardiovascular diseases or any other associated systemic disease were excluded by appropriate clinical and laboratory investigations.

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 Sickle cell clinic.

Details of all patients including demographic features, symptoms, details of crises, admissions/year, number of transfusions/year, any evidence of hematuria, oliguria were recorded. Detailed clinical examination including growth status (wt. and height), evidence of hypertension and any other significant findings were noted.

**Laboratory analysis**

The following laboratory information were collected and analyzed:

1. Serum creatinine, urea and electrolytes were determined using XIMOLA auto-analyser manufactured by RANDOX.

2. Estimated GFR was calculated using Schwartz formula: eGFR = K height/serum creatinine \((K = 0.33, \text{for full term babies}; K = 0.45, \text{for infants and children} > 1 \text{ year}, K = 0.55).\)

3. Routine urine examination for protein, and microscopy - About 10 ml of mid-stream urine was collected in universal sterile clear bottles for urinalysis. Young children were assisted by their accompanying parents/guardians for collecting the midstream urine, where they were instructed to wait a few seconds as the child starts voiding, then collect the urine. Single sample was collected for all the tests done during the study. Urine was analysed using PRIETEST eXP biochemistry analyser manufactured by ROBONIK.

4. Microalbuminuria-Microalbuminuria evaluation was done using Microalbuminuria testing kit - TURBILYTE MA manufactured by TULIP DIAGNOSTICS (P) Ltd., Goa.

TURBILYTE MA is used for semi-quantitative determination of albumin. It contains four solutions - ‘S’ for calibration, ‘R2’ Latex reagent, R1 Activation Buffer. Urine sample (1 ml) was mixed with R1 and R2 solution after calibrating the analyser with solution S. Results were obtained with the help of analyser. PRIETEST eXP biochemistry analyser manufactured by ROBONIK was used to interpret the results.

5. Renal Doppler and renal ultrasound was performed on a color flow Doppler machine, using 3.5-MHz sector probe. The patients were examined in the supine position: left lateral decubitus for the right kidney and right lateral decubitus for the left kidney. Doppler sonography was performed using the noncompression technique and under comfortable conditions. Doppler waveforms were obtained.

Renal dysfunction in this study was defined as the presence of at least one of these following criteria:

- The diagnosis of high eGFR (hyperfiltration) or low eGFR using Schwartz formula. \(^4\) Hyperfiltration was defined as a GFR greater than 140 ml/min/1.73 m\(^2\) and low GFR (Chronic renal failure) was described as GFR less than 80 ml/min/1.73 m\(^2\). \(^5\)
• Children were considered to have renal insufficiency if their total serum Creatinine concentrations 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 and or gross albuminuria when it was more than 300 mg/dl.  

• Renal Doppler Resistive Index (RI) >0.7 and Pulsatility Index (PI) >1.14 was considered abnormal
  a) Pulsatility index - A measure of the variability of blood velocity in a particular vessel, is equal to the difference between the peak systolic and minimum diastolic velocities and divided by the mean velocity during the cardiac cycle.
  b) Resistive index - A measure of pulsatile blood flow that reflects the resistance to blood flow caused by microvascular bed distal to the site of measurement.

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

There were total 110 homozygous (SS type) and 55 heterozygous (AS type) children between the age of 6 months to 15 years, who presented to the pediatric outpatient department (sickle cell clinic) or were admitted. There were 50 normal siblings/children (AA) in whom sickling test and hemoglobin electrophoresis was negative, they acted as controls.

There were 73 (66.36%) males and 37 (33.64%) females (Male:female ratio 1.97:1) among (SS type) and 36 (65.45%) males and 19 (34.55%) females (male:female ratio 1.89:1) among (AS type) children. While in normal children Male female ratio was 1.63:1. Majority belonged to lower and middle socioeconomic class.

Around 54% (59 patients) presented in steady state in sickle cell clinic and 46% (51 patients) presented in crisis state. Maximum patients in crisis were in age group above 10 years whereas in steady state majority were between 5-10 years (P >0.05). More children in SS type had chronic malnutrition (39%) as compared to acute malnutrition (18 %) (P <0.05).

During crisis 8 children (15%) had hematuria, 17 children (33%) had hypertension, there was no hematuria in steady state but around 1/3rd children (30%) had blood pressure near 90th percentile. There was no hematuria but 1 child (2%) had hypertension among AS type.

Mean per year transfusion rate was 2.53 in age group ≤5 years in comparison to 3.13 in age group 5.1 to 10 years and 3.68 in age group 10.1-15 years. Though this rise with age was statistically insignificant (P >0.05).

Vaso-occlusive crisis was most common (77%). Children in age group 10.1-15 years had maximum admissions in the hospital with mean per year of 6.05 in comparison to 4.84 in age group 5.1-10 years and 4.30 in age group of ≤5 years (P >0.05), though the difference was insignificant (P >0.05).

High serum urea was found in 12 patients (23.5%) in crisis state in comparison to only 2 (3.38) patients in steady state. Serum creatinine was high in 22 patients (43%) during crisis and in 9 patients (15%) during steady state. In AS type high serum urea was found in 2 patients (3.6%) and creatinine was high in 11 (13%).

A group of patients in crisis (20 pts., 39%), steady state (23 pts., 39%) and also in AS type (7 pts., 12.7%) had lower than normal serum creatinine. Few children showed same trend with urea also. These values mostly indicate hyperfiltration.

Abnormal urea and creatinine values (both above and below the range) were significantly more in children above 10 years. Mean urea and creatinine levels showed increase with age in both crisis and steady state. This trend was seen in AS group only with creatinine. Mean serum creatinine values of SS type below 5 years were significantly lower than AS group and normal children (Table 1).

Sodium abnormality was found in 44 (86%) patients in crisis state as compared to 41 (69%) in steady state (P <0.05). Hyponatremia was commoner than hypernatremia. In AS type 10 patients (18.18%) had sodium abnormality which was significantly less than other two groups.

Potassium abnormality was found in 20 patients (39%) in crisis state, 8 patients in steady state (13.5%) and 6 patients in AS type (11%). Hypokalemia was common in SS type and hyperkalemia in AS type.

High mean eGFR (203.08 ± 138.45) was seen in patients ≤5 years of age in crisis state and as age progressed there was fall. In steady state highest mean value (184.61 ± 74.78) was seen in age group 5.1-10 years and then there was fall .This trend of initial high mean eGFR which falls later, most probably indicates renal disease which is progressive and increases with age. Standard deviation which indicates magnitude of dispersion of value around mean also was found to be high where mean eGFR was high probably indicating different children in different stages of renal failure.
Table 1: Mean urea and creatinine values.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Mean serum urea (mg/dl)</th>
<th>Mean serum creatinine (mg/dl)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SS</td>
<td>AS</td>
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<tr>
<td></td>
<td>Crisis</td>
<td>Steady</td>
</tr>
<tr>
<td>≤5</td>
<td>29.66 ± 11.31</td>
<td>25 ± 4.32</td>
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<tr>
<td>5.1-10</td>
<td>28.93 ± 11.88</td>
<td>25.13 ± 4.87</td>
</tr>
<tr>
<td>10.1-15</td>
<td>38.85 ± 23.07</td>
<td>27 ± 9.49</td>
</tr>
<tr>
<td>Total mean</td>
<td>33.03 ± 17.40</td>
<td>25.66 ± 6.46</td>
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</table>

Significant increase in mean creatinine value after 10 years of age in SS and AS type both; same trend in SS type with mean serum urea to some extent.

Micro-albuminuria (62.75%) was more common than gross proteinuria (39.22%) during crisis (P <0.05) and in steady state (18.4% and 4%), (P <0.05). Micro-albuminuria was more common in crisis state (32 patients (62.75%)) than steady state [11 patients (18.64%)] (P <0.0001) and also in age group 5-10 years though insignificant (P >0.05). Micro albuminuria in AS type was commoner [14 patients (25.45%)] than steady state SS type. Among normal children, two patients had micro-albuminuria, but was febrile albuminuria (Figure 1).

In SS type micro-albuminuria was present in 26 (71%) patients out of 37 patients who had abnormal creatinine, whereas in 23% patients with normal creatinine (P <0.05). In AS type Micro-albuminuria was present in 45% patients who had abnormal creatinine.

Micro albuminuria was more common in children with severe and moderate anaemia (Hb <9g/dl) in crisis state (70%), steady state (33.33%) and AS type (62%) in comparison to mild anaemia.

In patients with lower eGFR, micro-albuminuria was present in 89.4% in crisis state, 50% in steady state and 62.5% in AS type. In cases of high eGFR, it was noted in 48% in crisis, 13% each in steady state and AS type. When eGFR was normal micro-albuminuria was less common (43% in crisis, 26% in steady and 22% in AS type). Many of these children had eGFR at upper normal limits indicating ongoing renal changes.

Ultrasound of kidneys was abnormal in 33 (30%) in SS type and 4 (5%) of AS type patients. Renal Doppler was abnormal in 48 patients (43.63%) in SS type and in 15 patients (27.27%) in AS type (P <0.05).

Majority of abnormal renal Doppler study was in the age above 5 years. In 37 patients with abnormal creatinine 29 (78.3%) had abnormal Doppler (P <0.05). Rest of the children with abnormal Doppler had lower values of creatinine. This indicates renal Doppler is a valuable non-invasive tool to detect renal involvement.

Renal Doppler and micro albuminuria were compared to find a better and earlier tool for detection of renal involvement. Abnormal renal Doppler (86% in crisis, 56% in steady, 54% in AS) was found in more number of children who had abnormal KFT (acute renal failure) than micro albuminuria (82.14% in crisis, 33% in steady, 45% in AS). This indicates that Renal Doppler may be a better tool for detection of renal involvement in sickle cell children followed up in sickle cell clinic. Moreover when renal functions are normal, renal Doppler and micro-albuminuria themselves are the indicators for renal involvement (Table 2).

Figure 1: Micro-albuminuria in crisis, steady state, AS, AA, patients.
**DISCUSSION**

In the present study the main aim was to document renal involvement in sickle cell disease and search for risk factors which can enhance the progression. Additionally an attempt is made to find non-invasive tests which can be used for screening in sickle cell follow up clinic. Renal parameters in SS type (crisis and steady state) and AS type children were compared with normal children.

Male preponderance seen in present study (1.97:1 in SS type and 1.89:1 in AS type) was also found by other authors who studied sickle cell children in Vidarbha region. Gladwin et al. commented that crises are less common in females as they have more fetal hemoglobin, moreover nitric oxide bioavailability and responsiveness is less in males which may be the cause for male preponderance in most studies. Nitric oxide reduces vaso-constrictor tone and hence thought to reduce vaso-occlusive crises.

Majority of patients in SS type were above 5 years (70%) and in AS type less than 5 years (28 pts., 50.91%). The reason for this age group distribution in SS type appears to be increased frequency of crises beyond toddler age and the reason for younger age in AS type may be early detection of siblings of homozygous children. Other studies in Vidarbha region also reported same distribution of ages.

We had more children with chronic malnutrition (39%). Archana Patel et al. found weight for age was affected in 96 % whereas stunting was in 86%. Our patients are regularly followed in sickle cell clinic which might be the reason for better nutrition.

Majority of our patients were from lower socioeconomic status and same was found by other authors of Vidarbha region. MSadat Ali et al. studied the effect of socioeconomic status on disease pattern and did not find any correlation, though he studied only bones and joints in his patients. We did not find any correlation of renal status with socioeconomic status but we had less children belonging to better social class.

As we wanted to study renal involvement in crisis and steady state separately we have categorically avoided same patients in both states. Parameters in steady states actually indicate the progression of renal involvement.

Asymptomatic hematuria is said to be common in both SS type and AS type and usually thought to be due to papillary necrosis. We found gross hematuria only during crisis in 8 children (15%). Additional 16% in crisis, 3% in steady state and 9% in AS group had microscopic hematuria.

Around 33% (17 children) in crisis had hypertension, 30% children in steady state and 1 child (2%) in AS type had blood pressure near 90th percentile. Hsein et al. found hypertension in 5.7% and borderline hypertension in 8.6% children. His patients included SS and SC type both. Around 60% of our patients with hypertension had microalbuminuria. Presence of hypertension may further increases the on-going renal involvement (and also stroke) in these children.

Vaso-occlusive crises, number of admissions and transfusions/year increased with age and were maximum above 10 years of age. Out of all types of vaso-occlusive crises 30% were abdominal, other studies also reported that around 30% patients presented with abdominal pain. More the number of abdominal crisis more are the chances for renal involvement.

High serum urea and serum creatinine levels were more during crises (23% pt. urea, 43% pt. creatinine) as compared to steady state (urea 3.3%, creatinine 15%) indicating reversibility of involvement after crisis. Mean urea and creatinine levels were more in crises 30% and 1 child (2%) in AS type.

A very interesting phenomenon seen in both states of SS type (crises and steady state) and also in AS type was serum creatinine lower than normal. Few children showed this trend with urea also. These kinds of values have been much discussed in literature in sickle cell

<table>
<thead>
<tr>
<th>KFT</th>
<th>Patient status - SS</th>
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<th>AS</th>
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<tbody>
<tr>
<td></td>
<td>Crisis</td>
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<td>Steady</td>
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<td></td>
<td>Doppler abnormal</td>
<td>Micro albuminuria abnormal</td>
<td>Doppler abnormal</td>
<td>Micro albuminuria abnormal</td>
<td>Doppler abnormal</td>
<td>Micro albuminuria abnormal</td>
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<tr>
<td>Abnormal</td>
<td>24 (85.7%) (n=28)</td>
<td>23 (82.14%) (n=28)</td>
<td>5 (55.5%) (n=9)</td>
<td>3 (33.33%) (n=9)</td>
<td>06 (54.54%) (n=11)</td>
<td>05 (45.4%) (n=11)</td>
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<tr>
<td>Normal</td>
<td>10 (43.4%) (n=23)</td>
<td>9 (39.19%) (n=23)</td>
<td>9 (18%) (n=50)</td>
<td>8 (16%) (n=50)</td>
<td>09 (20.45%) (n=44)</td>
<td>09 (20.45%) (n=44)</td>
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</table>
anemia and are thought to be because of the proximal tubular dysfunction and hyperfiltration. Most authors think as these values indicate renal damage should not be ignored.

In this study children with lower than normal creatinine were maximum in age group ≤5 years, after this age number of children with high values increased. This appears to be directly related to high GFR, early in life hyperfiltration leads to lower than normal values of urea and creatinine but with age hyperfiltration causes permanent renal changes and damage to glomeruli leading to decrease in GFR and increase in serum urea and creatinine. High mean GFR in early age has been reported by other authors also. Estimated GFR is high most probably because of increased renal vasodilation due to chronic anemia leading to hyperperfusion and hyperfiltration. Anigilage and Adenibeg  attributed finding of high GFR to error in urine collection and intravenous hydration which is a part of vaso-occlusive crisis management. In the present study low values also are found in steady state where there was no question of hydration. Anigilage like present study also reported reversibility of eGFR to normal during steady state reducing the number of children with low values.

Abnormal sodium specially hypo-natremia was more common in both crisis (86%) and steady state (69%). Mean serum sodium also was much lower in both states of SS type as compared to AS type children. Mean potassium values were higher in both states of SS type and even in AS type as compared to normal children. Similar electrolyte abnormalities have been described by Agoreyo et al. also.

Urinary concentration capacity is described to be the first parameter recognised to be affected (hypothenuria) in sickle cell anemia which could be restored after adequate multiple transfusions till age of 10-15 years. This may not happen after age of 15 years. These irreversible changes indicate progressive renal damage with age. We could not study this parameter.

Micro-albuminuria has attracted attention of many authors as it may be an “easy to measure” parameter to detect early renal involvement as well as, it may have role in progressive renal deterioration due to hyperfiltration injury. We also found this parameter useful, as a large number of children with low GFR and abnormal urea and creatinine level and even some children with high GFR and lower than normal urea and creatinine had micro-albuminuria indicating its usefulness in early detection of renal involvement in both SS group and AS group.

Renal Doppler is second non-invasive test for early detection of abnormal renal function. Increased pulsatility and resistive indices have been reported to be early imaging indicators of damage. The disadvantage of cost and availability maybe the demerits of renal Doppler. We found it better tool than micro-albuminur. Ultrasound which is cheaper has not been found very useful in the present study 70% SS group children had normal kidneys. Mohanty and Narayan also had similar observations in their patients.

To conclude the renal involvement starts below 5 years and increases with age. Males out number females in renal involvement may be due to more crises. Homozygous children specifically in crises are affected more. Renal involvement is more in children with moderate and severe anaemia. Hence increasing age, male sex, homozygous state and severe anaemia appear to be risk factors for renal involvement.

High GFR and lower than normal urea and creatinine values also should be alert the paediatrician about renal involvement. Renal Doppler and microalbuminuria can be used as screening methods in sickle cell clinic as they indicate renal involvement in presence or absence of other features. Renal Doppler appears to be superior.

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