ORIGINAL RESEARCH

Disease severity and renal function among sickle cell anaemia patients in a tertiary hospital, South-south, Nigeria: a cross sectional study

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Abstract

Background

Renal disease is a recognized complication of sickle cell anaemia (SCA), especially from the third decade of life and is linked to disease severity. This study assessed the association between disease severity and renal function among SCA patients using routine and newer markers of renal function.

Methods

This cross-sectional study recruited 85 SCA patients. Disease severity was assessed using modified Adegoke criteria which include the frequency of transfusion, painful crises, packed cell volume, and history of complications such as hypertension and chronic leg ulcers. Renal function was assessed using urea, creatinine, and beta-2-microglobulin (β2-M). Association was determined between renal function and disease severity using Pearson's correlation. P-value < 0.05 was taken as significant.

Results

The mean age of participants was 27.2 ± 7.6 years with 41(48.2%) males and 44 (51.8%) females. The mean packed cell volume, serum creatinine, serum urea, and β2-M were 24.0± 4.1%,17.6±7.5 mg/dL, 0.7±0.3mg/dL, 3.4±1.2mg/l respectively. A majority (54.1%) of them had a mild disease while 35.3% and 10.6% had moderate and severe diseases, respectively. Forty of the SCA patients had urine specific gravity below 1.010. The mean values of systolic blood pressure (p=0.001) diastolic blood pressure (p=0.001), serum creatinine (p=0.028) and β2M (p=0.019) significantly increased with disease severity. There was a significant positive correlation between SCA disease severity and serum urea (r=0.229; p=0.035), and serum β2-microglobulin (r=0.270; p=0.012).

Conclusion

Sickle cell anaemia severity is associated with a decline in renal function using both traditional and novel renal markers. Serum β2-M may serve as a useful marker of renal function and disease severity in SCA

Keywords: Sickle cell anaemia, beta-2-microglobulin, disease severity, renal function

Background

Sickle cell anaemia (SCA) is a chronic sickling disease characterized by clinical events called crisis[1]. These clinical events are modified by factors such as hydration, de-oxygenation, temperature, pH, viscosity, levels of haemoglobin F, and co-existing haemoglobinopathies such as thalassemia and glucose-6-phosphate dehydrogenase deficiency[2].

Individuals with SCA are at risk of developing renal disease due to chronic sickling underlying the disease and resulting in hemolysis-induced renal injury[3]. Chronic kidney disease (CKD) is a recognized complication of SCA associated with risk factors such as hypertension, low haemoglobin concentration, hemolysis, prior vaso-oclusive crisis, BS gene haplotype[4-9]. Renal involvement contributes substantially to reduced life expectancy in patients with SCA, accounting for 16-18% mortality[10].

Manifestations of renal complication of SCA include asymptomatic or symptomatic albuminuria or proteinuria. Sklar et al[11] reported renal insufficiency in 4.6% of 116 SCA patients that was significantly associated with proteinuria and increased age. In Nigeria, 50% of 72 patients with SCA were reported to have albuminuria[12], while proteinuria was found in 41% of 73 SCA patients in Saudi Arabia where about 27% of SCA patients were within the first three decades of life[13-14]. However, in the USA, less than 1% of the total population of 375, 152 SCA patients studied had renal failure[15]. Powars et al[4] reported that 4.2% of 725 SCA patients had irreversible renal damage progressing to end-stage renal failure.

Chronic kidney disease has been identified as the major risk factor for early mortality in adult patients with SCA[16]. Previous studies have reported the significant burden of renal complications frequently encountered among patients with SCA[11-15]. However; local reports are limited despite large number of sickle cell disease patients in Nigeria.

https://dx.doi.org/10.4314/mmj.v35i1.3

The rising prevalence of renal impairment in SCA may be due to increased survival, co-morbidities such as uncontrolled hypertension, diabetes mellitus16and analgesic abuse for chronic pain that characterize the disease. The kidney in SCA individuals is affected by both the hemodynamic changes of chronic anaemia and the consequences of recurrent vaso-occlusion leading to structural and functional changes and progression to CKD[17].

Despite the use of the disease severity scoring system (DSSS) to categorize patients and routine screening for renal impairment using creatinine, there are limited studies establishing the relationship between the disease severity and early renal disease. This may be due to the insensitivity of serum creatinine for early renal function decline based on the influence of body mass index (BMI) and hyper-filtration present in patients in SCA. It is, therefore, necessary to assess any relationship between disease severity and renal function using other renal markers such as β2-microglobulin. This study assessed the disease severity of SCA patients and determined its association with renal function using both traditional and new markers of renal function in a tertiary hospital in Southern Nigeria.

Materials and Methods

Study Design

This was a cross-sectional study carried out in the Departments of Haematology and Chemical Pathology of the University of Benin Teaching Hospital (UBTH), Benin City, Edo State over a six-month period. This is a tertiary hospital, located in Southern Nigeria and serves as a major referral center to the neighbouring states of Ondo, Kogi and Delta. The sickle cell clinic is run by consultant haematologists and an average of 40 SCA patients are seen weekly in the consultant outpatient clinic.

Study Sample Size

The sample size was calculated using the formula for a cross-sectional study18. N = Z2P (1-P) / d2 where N = minimum sample size, Z = normal standard deviation at 95% confidence interval = 1.96, P = proportion of the SCA patient with sickle cell nephropathy was 5% from a previous study[15]. d = degree of precision = 5%. This formula gave a minimum sample size of 80 after including a 10% attrition rate.

Study Participants

The study population included 85 adult HbSS patients diagnosed by haemoglobin electrophoresis who were attending the haematology clinic of UBTH. Inclusion criteria were consenting HbSS patients of age ≥18 years, in steady state and without established chronic kidney disease. HBSS patients on cimetidine or trimethoprim, haematological malignancies, on-going infection and diabetes mellitus were excluded from the study.

Data Collection Tool

The self-administered questionnaire was used to obtain information on demography, medical history, history of the previous transfusion, and chronic complications such as hypertension and chronic leg ulcer in the last 12 months. The height and weight of each participant were measured using a stadiometer and weighing scale in meters and kilogram (Kg) respectively, to derive the BMI in Kg/m^2. To measure the weight, participants were allowed to remove shoes and any heavy clothing before mounting the scale and heads kept in upright position after which the readings taken in kilogram (Kg) by the investigator. Two readings were taken for each participant and the average was recorded. Height was taken using a standard measuring meter attached to the scale, participants were asked to remove head ties and caps before applying the measuring ruler close to the crown of the head. Blood pressure was measured using an Accouson sphygmomanometer. Two readings at 30-minute intervals were taken for each participant and the mean value was recorded in mmHg.

Ten (10) mL of venous blood was collected from the antecubital fossa of each patient and 3 mL was dispensed into EDTA bottle for hematocrit while 7 mL was dispensed into plain tubes and allowed to clot. Serum samples were harvested following centrifugation at 1, 500g over 15 minutes for biochemical assays to assess renal function. Urea and creatinine concentrations in serum were determined using Urease and Jaffe kinetic methods (Randox kits) respectively while β^2M was determined using ELISA (Quantikine kit) method. The absorbances for urea and creatinine were determined by Spectrumlab PC 22 Spectrophotometer and β^2M using a microplate reader. Thereafter, 5 mL of urine was collected for specific gravity using a dipstick. Known concentrations of quality control sera were assayed for quantitative parameters while qualitative control was used for urine dipstick. Hyposthenuria is defined as urine-specific gravity less than 1.010[19].

A modification of the disease severity scoring system (MDSSS) by Adegoke et al20 was used to stratify the subjects into mild, moderate and severe disease groups. Parameters used for scoring were stable packed cell volume (PCV), frequency of painful crises, and transfusion history within the previous 12 months as well as presence or absence of complications such as hypertension, chronic leg ulcer, cerebrovascular accident (CVA), and acute chest syndrome. The minimum score was 3 while the maximum score was 12. SCA patients were then categorized into mild, moderate and severe disease groups using parameters proposed Adegoke et al[20].

Statistical Analysis

Data obtained were entered analyzed using the statistical package of social sciences (SPSS) software version 20. Descriptive statistics were represented with tables. Categorical variables were compared using Chi-square test. The comparison between group means was done using student t- test. Comparison of mean values among three groups was done using ANOVA. Tukey HSD (Honest Significant Difference) was used for post hoc analysis. The p value < 0.05 was taken as the cut off level for significance. Pearson's correlation test was used to test association between continuous variables.

Ethical Approval and Consideration

Ethical approval was obtained from Human Research and Ethical Committee of UBTH. The reference number of the approved protocol was ADM/E 22/A/VOL.VII/1038.

Informed consent was obtained from all participants in the study. The study did not involve any therapeutic trials. Confidentiality of the provided information was ensured throughout the study.

Results

Table 1: Demographic Information and Clinical Parameters of Study Participants

	SCA (n = 85)	Controls (n= 31)	P value
Age (years)	27.2 ± 7.6	29.4 ± 6.8	0.159
Sex			
Male	41 (48.2)	19 (61.3)	0.213
Female	44 (51.8)	12 (38.7)	
BMI (kg/m^2)	17.9 ± 4.8	24.1 ± 3.7	<0.001
<18	50 (58.8)	1 (1.2)	
18 – 24.9	32 (37.6)	17 (54.9)	
25 – 29.9	2 (2.4)	11 (35.5)	
30 – 34.9	0 (0.0)	2 (2.4)	
>35	1 (1.2)	0 (0.0)	
Systolic Blood Pressure (mm/Hg)	109.3 +13.9	114.5+10.3	0.58
Diastolic Blood Pressure (mm/Hg)	67.1+10.7	77.2+9.1	<0.001

Table 2: Comparison of Haematocrit and Biochemical Parameters of Study Participants

	SCA	Controls	P-value
Packed Cell Volume (%)	24.0+ 4.1	45.7+5.8	<0.001
Urea (mg/dL)	17.6+7.5	24.0+8.0	<0.001
Creatinine (mg/dL)	0.7+0.3	0.9+0.3	0.016
β_2-microglobulin (mg/l)	3.4+1.2	2.3+0.7	<0.001

Table 3: Frequency of Disease Severity Categorization and Some Severity Indices

Disease Severity (n = 85)	Frequency (n)	Percentage (%)
Mild	46	54.1
Moderate	30	35.3
Severe	9	10.6
Hypertension	4	4.7
Ulcer	16	18.8
Transfusion	71	83.5
Painful crises(number of episodes)	65	76.5
1	21	24.7
2	17	20
3	13	15.3
4	6	7.1
5	7	8.2
6	1	1.2

https://dx.doi.org/10.4314/mmj.v35i1.3

Table 4: Blood Pressure and Biochemical Parameters Based on Disease Severity

	Mild	Moderate	Severe	P-value
Age (years)	26.5+8.1	27.0+5.9	33.8+8.7	0.174
Body Mass Index (Kg/m^2)	17.7+4.1	17.1+2.5	19.5+2.6	0.27
Systolic Blood Pressure (mmHg)	108.2+14.6	109.5+11.6	116.3+19.8	0.001
Diastolic Blood Pressure (mmHg)	66.0+11.9	67.7+8.8	71.7+7.5	0.004
Urea (mg/dL)	16.8+8.8	17.9+5.5	22.7+4.1	0.058
Creatinine (mg/dL)	0.7+0.4	0.7+0.2	1.1+0.3	0.028
β_2-microglobulin (mg/l)	3.3+1.0	3.4+1.0	4.6+2.7	0.019

Table 5: Post Hoc Analysis

Parameter			P value
SBP	Mild disease	Moderate disease	0.543
		Severe disease	0.000*
	Moderate disease	Severe disease	0.001*
DBP	Mild disease	Moderate disease	0.400
		Severe disease	0.001*
	Moderate disease	Severe disease	0.006*
Creatinine	Mild disease	Moderate disease	0.857
		Severe disease	0.011*
	Moderate disease	Severe disease	0.011*
β_2-microglobulin	Mild disease	Moderate disease	0.429
		Severe disease	0.005*
	Moderate disease	Severe disease	0.025*

Table 6: Correlations of variables and disease severity in SCA subjects

		Systole	Diastole	BMI	Urea	Creatinine	β_2M	Severity
Age	R	0.158	0.162	0.384	0.259	0.360	0.108	0.162
	P	0.149	0.140	0.000*	0.017*	0.001*	0.325	0.138
Systolic BP	R	-	0.510	0.276	0.256	0.174	0.195	0.333
	P	-	0.000*	0.011*	0.018*	0.111	0.074	0.002*
Diastole BP	R	0.510	-	0.219	0.152	0.96	0.178	0.313
	P	0.000*	-	0.044*	0.165	0.006*	0.103	0.003*
BMI	R	0.276	0.219	-	0.213	0.365	0.006	0.046
	P	0.011*	0.044*	-	0.05	0.001*	0.956	0.679
Urea	R	0.256	0.152	0.213	-	.422	.336	.229
	P	0.018*	0.165	0.05	-	0.000*	0.002*	.035*
Creatinine	R	0.174	0.96	0.365	.422	-	.409	.198
	P	0.111	0.006*	0.001*	0.000*	-	0.000*	.070
β_2M	R	0.195	0.178	0.006	.336	.409	-	.270
	P	0.074	0.103	0.956	0.002*	0.000*	-	0.012*

BP (blood pressure), BMI (body mass index), β_2M (β_2-microglobulin)

https://dx.doi.org/10.4314/mmj.v35i1.3

A total of 85 SCA patients with a mean age of 27.2 ± 7.6 years were studied. They comprised 41(48.2%) males and 44 (51.8%) females. The majority (58.8%) of the study participants had BMI values less than 18.0 kg/m2 while 32 (37.6%) had a BMI of 18.0– 24.9 kg/m^2. The mean systolic and diastolic BP were 109.3 ±13.9 and 67.1±10.7 respectively. The mean packed cell volume serum creatinine, serum urea, and β2-microglobulin were 24.0± 4.1%,17.6±7.5 mg/dL, 0.7±0.3mg/dL, 3.4±1.2mg/l respectively. Forty (47.1%) of the study participants had urine-specific gravity below 1.010. (Table 1)

Based on the proposed MDSSS, the majority (54.1%) of the SCA patients had a mild disease while 35.3% and 10.6% of subjects had moderate and severe diseases respectively. Seventy-one (83.5%) of the SCA patients had a positive history of previous blood transfusion while 76.5% had a history of vaso-occlusive crises in the preceding 12 months. History of complications such as chronic leg ulcer and hypertension was present in 18.8% and 4.7% of patients respectively. (Table 2)

The mean values of systolic blood pressure (p=0.001) diastolic blood pressure (p=0.001), serum creatinine (p=0.028), and β2M (p=0.019) significantly increased with disease severity. (Table 3)

Post-hoc analysis showed that the mean serum β2-microglobulin was significantly higher in SCA patients with severe disease compared with those with moderate disease (p=0.025) and mild disease (p=0.005). It also showed the mean serum creatinine in SCA was significantly higher in those with severe disease compared to those with mild disease (p=0.011) and moderate disease (p=0.011). (Table 4). There was no significant difference in the mean SG of urine across the disease severity. (Table 4)

There was a significant positive correlation between SCA disease severity and serum urea (r=0.229; p=0.035), and serum β2-microglobulin (r=0.270; p=0.012). (Table 5)

Discussion

The participants in this study were majorly young adults with a mean age of about 27 years. This is similar to 24 years reported as the mean age of the adult SCA population in a previous study from Nigeria[11]. This may reflect the lower life expectancy of SCA patients compared to individuals who do not have SCA. Severe disease was found in 10.6% of our study participants, similar to 10.4% reported by Adegoke et al[20]. However, this finding is higher than 4.5%, 5.8%, 7.8% reported in Saudi Arabia, Yemen and Darkar respectively[21-23]. The differences in the prevalence of severe disease in these various geographical locations could be explained by the role of genetic factors like β-globin haplotype polymorphisms. The predominant haplotype in Saudi Arabia and Yemen is the Arab-Indian haplotype which is associated with mild disease. Whereas, the Benin haplotype that is predominant in Southern Nigeria has a severe clinical presentation[20,24-26]. The use of certain medications such as hydroxyurea by some SCA patients in the various studies and other genetic abnormalities such as glucose-6-phosphate dehydrogenase deficiency, thalassemia, and fetal haemoglobin may also modify the clinical presentation of SCA[2]. In addition, the difference in methodology such as the age of study participants, and parameters used in the assessment of disease severity may also partly explain the variation in the disease severity in these studies.

The mean values of serum urea and creatinine in our study participants are close to the lower limit of normal reference values for Nigerians[27,28]. These traditional markers of kidney function may be affected by non-renal factors which may limit their use[26]. For example, muscle mass affects serum creatinine while the amount of protein intake and the body's ability to catabolize urea may affect serum urea[29]. SCA patients usually have reduced muscle mass which is supported in our study where about 60% were underweight. This has implications for clinical practice because serum creatinine may still be within the normal reference values in SCA patients even in the presence of significant kidney damage[29]. This underscores the need to use other markers of kidney function that are not affected by reduced muscle mass.

The mean value of serum β2-microglobulin level in our study participants is higher than the upper limit of normal reference value for Nigerians[30]. The finding in this study is similar to the report by De Jong el al[31]. β2-microglobulin is a more favourable marker that could be used to assess renal function in SCA patients[32]. It is a low molecular weight protein and the component of the major histocompatibility complex I[33-35]. It measures about 12,000 Dalton and is produced by nucleated cells[33-35]. It is filtered at the glomerulus, almost completely absorbed and destroyed by the proximal convoluted tubules[33-35], hence only a minimal amount is seen in the urine of healthy individuals. Measurement of β2-microglobulin can therefore give information on both glomerular and tubular function. Autoimmune disorders and certain malignancies may lead to increase serum β2-microglobulin, hence these conditions must be excluded when using it to assess renal function.

The tubular injury occurs early in sickle cell nephropathy before glomerular function becomes impaired[36], therefore, markers of tubular injury will be highly valuable in the detection of early renal dysfunction even when serum creatinine is within normal limits. Although a detailed assessment of tubular function was not done, urine specific gravity which also assesses concentrating ability of the renal tubules was done. The result showed the impaired concentrating ability of the renal tubules in about half of the SCA patients. The mean specific gravity of 1.007 found in this study is similar to the 1.006 reported in a study done in Cameroon[37]. One of the advantages of using β2-microglobulin in SCA patients over traditional renal markers such as urea and creatinine is that it assesses both the glomerular and tubular function which are commonly affected in sickle cell nephropathy.

The limitation of this study was that we did not include a reference exogenous renal marker such as inulin for comparison with β2-microglobulin in the assessment of renal function among SCA because the methodology involved is very cumbersome. Also, a detailed assessment of tubular function was not done in the study due to limited funds.

Conclusion: Majority of patients with sickle cell anaemia had mild to moderate disease using the MDSSS. The disease severity was significantly associated with declining renal function using both traditional and novel renal markers; however, serum β2-microglobulin had a better association with disease severity than creatinine. Serum β2-microglobulin may serve as a useful marker of renal function and disease severity in patients with SCA especially when serum creatinine is within normal limits.

https://dx.doi.org/10.4314/mmj.v35i1.3

Conflict of Interest

None

Source of funding

Self

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