Important clinical and laboratory correlates of glomerular filtration rate in sickle cell anemia

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Abstract

Background: Renal impairment is routinely assessed using the estimated glomerular filtration rate (eGFR) and it may be helpful to obtain certain clinical or laboratory markers, which show relationship with glomerular filtration rate (GFR) in sickle cell disease (SCD).

Aim: To assess the relationship between important clinical and laboratory parameters in SCD, and the eGFR.

Patients and Methods: Steady state clinical and laboratory data were obtained from 228 homozygous SCD patients seen over a 7-year period. The GFR was estimated using (isotope dilution mass spectrometry) traceable modification of diet in renal disease (MDRD) and Cockroft–Gault methods. The correlation coefficient and independent *t*-test were done to assess the level of significance between the eGFR_MDRD and the known indicators of disease severity.

Results: The serum alkaline phosphatase (ALP) and serum direct bilirubin levels both showed significant inverse relationship with eGFR_MDRD $P = 0.012$ and $P = 0.24$, respectively. The patients' age, Hb, leukocyte count, platelet count, serum direct bilirubin and aspartate transaminase did not show a significant correlation. The eGFR_MDRD was more discriminatory revealing that 20.3% of the patients had suboptimal GFR. Proteinuria in steady state was observed in 20.3% of the patients.

Conclusion: High serum direct bilirubin and ALP are associated with a deteriorating eGFR_MDRD. Other clinical and laboratory indicators of disease severity in SCD do not show the relationship with the GFR. MDRD_GFR estimation seemed to be a more appropriate method of estimating GFR in SCD.

Key words: Cockroft–Gault, glomerular filtration rate, modification of diet in renal disease sickle cell

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Introduction

Sickle cell anemia (SCA) is associated with chronic end organ complications, which tend to be more prevalent with improved patient care and longer survival. Renal failure due to sickle nephropathy is a documented cause of increased morbidity and mortality, affecting 12–21% of adult sickle cell patients.[1,2] There are varied pathogenetic mechanisms for glomerular changes seen in sickle cell disease (SCD). These include mesangial phagocytosis of sickled red cells, immune complex glomerulonephritis, hyperfiltration leading to glomerular injury and hypertrophy.[3] These effects are primarily derived from the basic pathological process of the disease, which involves red cell sickling with subsequent episodic ischemia of the end organs.[4]

The occurrence of renal impairment generally and hyperfiltration, in particular,[5] has been previously associated with laboratory indicators of hemolysis in this group of patients.[6] Progression of sickle nephropathy can

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be prevented by routine use of angiotensin-converting enzyme inhibitors in susceptible patients – those with micro-albuminuria.[7] Sickle nephropathy is usually heralded by micro-albuminuria (proteinuria) in most instances. However nephropathy also seems to occur in patients with severe form the disease. Recent studies have implicated the depletion of nitric oxide as a result of increased hemolysis, as part of the pathogenetic mechanism underlying some chronic complications of the disease.

Estimated glomerular filtration rate (eGFR) is usually utilized in the clinical setting to assess renal status, as well as grade impairment of renal function. Several methods of estimating glomerular filtration in sickle cell patients have been proposed as a result of hyperfiltration occurring in a number of these patients.[8-10] However, there is no current agreement as to the methods to be used by all physicians in this patient group.[10] The Cockroft–Gault (eGFR_CG) and the modification of diet in renal disease (eGFR_MDRD) has been used in several studies and will be employed in this study.

In the clinical setting, apart from proteinuria and estimation of GFR, presence of other clinical and laboratory features may correlate positively with nephropathy.[2] These features may serve as early indicators of nephropathy, prompting the physician to further investigate for its presence. This is particularly important because the presence of kidney disease adversely affects survival in sickle cell, and early detection is currently advocated. Assessment of the relationship between known clinical and laboratory indicators of disease severity and the GFR in homozygous sickle cell patients may provide insight into possible predictive indices, for sickle cell associated renal impairment.

This study aims to evaluate the relationship between the eGFR and important clinical and steady state laboratory parameters in homozygous sickle cell patients. It was also aimed at comparing the eGFR using MDRD and CG method in this patient population.

Patients and Methods

This is a retrospective study of patients who had been diagnosed with SCA (homozygous SCD) and were being seen as out-patients at the University of Nigeria Teaching Hospital (UNTH), from July 2006 to November 2014. Ethical approval was obtained from the UNTH Health Research and Ethics Committee and informed consent was obtained from the patients. Information obtained from medical records of patients included demographic data; age, sex, frequency of crises per annum, as well as steady state laboratory indices; hemoglobin (Hb) concentration, white cell and platelet counts, aspartate transaminase (AST), alkaline phosphatase (ALP) and direct bilirubin assays.

The serum creatinine and liver enzymes were measured using the enzymatic method in the Reflotron plus chemistry analyzer. Those who had acute kidney injury and who took drugs that may affect creatinine were excluded from the study.

The eGFR_CG was calculated using the formula;

([140-age] [weight in kg] [0.85 if female])/(72 × serum creatinine [in mg/dL]),

While the isotope dilution mass spectrometry (IDMS)-traceable MDRD study equation (for creatinine methods calibrated to an IDMS reference method), was calculated using the formula;

$$GFR\ (mL/min/1.73\ m^2) = 175 \times (S_{cr})^{-1.154} \times (age)^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American}).$$

The equation does not require weight or height variables because the results are reported normalized to 1.73 m^2 body surface area, which is an accepted average adult surface area.

Normal renal function GFR = 100–130 (for females) or 100–140 (for males)/min/1.73 m^2.

- Stage 1: Slightly diminished function; kidney damage with normal or relatively high GFR (≥90 mL/min/1.73 m^2). Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies[11]
- Stage 2: Mild reduction in GFR (60–89 mL/min/1.73 m^2) with kidney damage. Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies[11]
- Stage 3: Moderate reduction in GFR (30–59 mL/min/1.73 m^2).[11] British guidelines distinguish between stage 3A (GFR 45–59) and stage 3B (GFR 30–44) for purposes of screening and referral
- Stage 4: Severe reduction in GFR (15–29 mL/min/1.73 m^2)[11] Preparation for renal replacement therapy
- Stage 5: Established kidney failure (GFR <15 mL/min/1.73 m^2, permanent renal replacement therapy,[11] or end stage renal disease.

Statistical analyses

The relationship between the clinical and laboratory parameters and eGFR_MDRD was then evaluated using the Pearson correlation coefficient (two-tailed) for the numerical variables and the Spearman correlation coefficient (two-tailed) for the nominal and ordinal variables. Significance was set at all values less 0.05, independent *t*-test for comparing the equality of means, without assuming equal variance was done to determine the significance of the differences observed in the various

sexes. The Durbin–Watson logistic regression was also done for analysis of variance. The Statistical Package for Social Sciences (SPSS) version 17.0 (Chicago, IL, USA, 2009) was used for data analysis and the results obtained were expressed in figures and tables.

Results

Two hundred and twenty-eight patients were studied 144 males (63.2%), 84 females (36.8%). Their median age was 24 years, and their ages ranged from 6 to 55 years. A suboptimal eGFR_MDRD was observed in 20.3% of the patients. Proteinuria was observed in 20.3% of the patients in steady state. The mean eGFR_CG was 111.4 ± 50.3 mL/min/1.73 m^2 while the median was 98 mL/min/1.73 m^2, (n = 60). The mean eGFR_MDRD was 150 ± 74.8 mL/min/1.73 m^2, with a median of 132.7 mL/min/1.73 m^2 (n = 72). Table 1 shows the stages of normalcy or renal impairment observed in the patients. The eGFR_CG seem to underestimate the GFR and 51.7% of the patients had sub-normal eGFR compared to 20.3% obtained using MDRD equation. Step-wise analysis of variance revealed that only the gender of the patient had any relationship with the eGFR_ MDRD (f = 9.251, P = 0.004).

Table 1: Distribution of homozygous sickle cell patients according the staging of renal impairment

	Number of patients in group/ percentage	
	Cockroft-Gault GFR	Modification of diet in renal disease GFR
Normal (GFR>100 mL/min/1.73 m^2)	29 (48.3)	55 (79.7)
Stage 1 (90-99 mL/min/1.73 m^2)	7 (11.6)	4 (5.8)
Stage 2 (60-89 mL/min/1.73 m^2)	19 (31.7)	8 (11.6)
Stage 3 (30-59 mL/min/1.73 m^2)	4 (6.7)	2 (2.9)
Stage 4 (15-29 mL/min/1.73 m^2)	1 (1.7)	0
Stage 5 (<15 mL/min/1.73 m^2)	0	0

GFR=Glomerular filtration rate

The median values of the laboratory indices observed were; white blood cell count – 11.0 × 10^9/L, Hb concentration – 7.8 g/dL, platelet count – 330 × 10^9/L, direct bilirubin 12.4 μmol/L, AST – 17 μmol/L and median frequency of crises – 2 episodes/annum. Figure 1a shows a dot plot of the patients' serum bilirubin, eGFR_MDRD in relation to their proteinuria status, this does not reveal any apparent relationship between these parameters. While Figure 1b shows a dot plot of the relationship between some established markers of disease

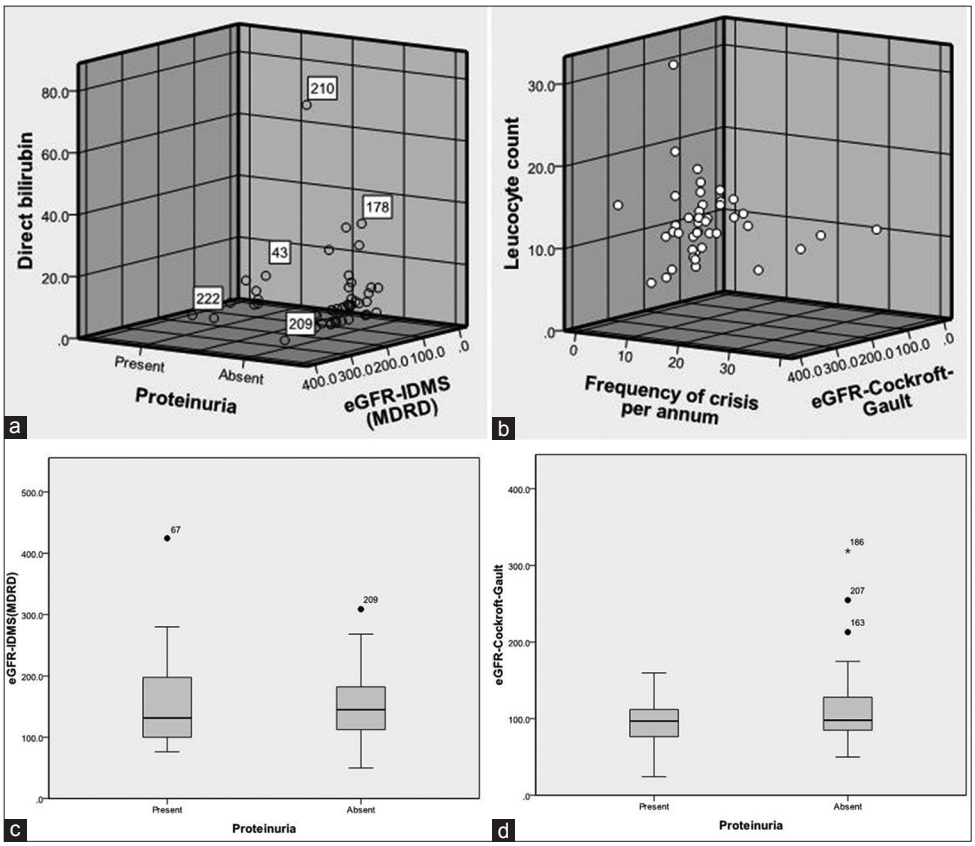

Figure 1: (a) Distribution of patients' serum direct bilirubin and glomerular filtration rate (GFR) (modification of diet in renal disease [MDRD]) in those with and without proteinuria. (b) Boxplot of white cell count, frequency of crises and GFR (Cockroft–Gault [CG]) in sickle cell anaemia patients. (c) MDRD estimation of GFR (eGFR) in sickle cell patients with and without proteinuria. (d) CG eGFR in sickle cell patients with and without proteinuria

Table 2: Gender distribution of clinical and laboratory features of SCA patients

	n	Mean	Median	Correlation coefficient with eGFR_(IDMS) MDRD (*P*)	*P* (*t*-test: Males vs. females)
Age (years)					
Male	144	25.1	24	0.085 (0.476)	0.667
Female	84	25.5	24		
Frequency of crisis (per annum)					
Male	87	4	2	0.04 (0.769)	0.672
Female	51	4	2		
Haemoglobin concentration (g/dL)					
Male	99	7.8	7.8	0.185 (0.14)	0.38
Female	66	7.6	7.6		
White cell count ($\times 10^9$/L)					
Male	115	13.1	11.2	0.011 (0.928)	0.995
Female	78	12.4	10.8		
Platelet count ($\times 10^9$/L)					
Male	104	346	323	0.099 (0.424)	0.435
Female	72	361	334		
Serum direct bilirubin					
Male	67	15.2	8.6	−0.285 (0.024)*	0.833
Female	44	22.1	10.2		
Serum aspartate transaminase					
Male	66	21.9	17	−0.102 (0.436)	0.526
Female	42	20.3	14.5		
Serum alkaline phosphatase					
Male	66	76.5	58.5	−0.319 (0.012)*	0.744
Female	42	73.0	64.5		
eCG_GFR					
Male	40	114	98	0.845 (0.001)*	0.501
Female	20	106	101		
e (IDMS) MDRD_GFR					
Male	46	159	167	Not admissible	0.030*
Female	23	130	108		

*Significant values $P<0.05$. eCG_GFR=Estimated glomerular filtration rate using Cockroft-Gault formula; eGFR_MDRD=Estimated glomerular filtration rate using modification of diet in renal disease formula; SCA=Sickle cell anaemia; IDMS=Isotope dilution mass spectrometry

severity – leucocyte count, frequency of crisis per annum and the eGFR_CG. These do not show any observable trend in the relationship between the GFR (either MDRD or CG) and some indicators of severe SCD. However the box plot on Figure 1c indicates that patients who had proteinuria seemed to have a lower eMDRD_GFR compared to patients in whom it was absent. The eGFR_CG did not indicate any obvious relationship between proteinuria – Figure 1d.

The serum ALP was shown to have a significant negative correlation with the eGFR_MDRD, $P = 0.012$, and there was no significant difference in this parameter between male and female patients [Table 2]. This indicates that higher levels of serum ALP are associated with lower GFR in sickle cell patients. Furthermore, the serum direct bilirubin (an indicator of the rate of hemolysis), also showed a significant inverse relationship with the eGFR_MDRD, $P = 0.024$. The GFR calculation using the MDRD and CG methods also showed significant positive correlation, as expected, and notably there was no significant difference in the values obtained for both males and females [Table 2].

Other clinical and laboratory parameters including patients' age, frequency of crisis per annum, Hb concentration, leucocyte and platelet counts, serum direct bilirubin and AST, did not show any significant relationship with the eGFR_MDRD.

Discussion

The occurrence of renal impairment in SCD is known to increase with age,[7] the median age of the patients studied was 24 years, and overt clinical nephropathy is usually been evident at this stage. Several studies done previously reported the frequency of renal impairment to be between 4–18%.[7,9] However, renal impairment was observed in 20.3% of the patients in our cohort while chronic kidney disease (CKD) requiring renal replacement was observed in 1.7% of the patients. Also, previous research had shown glomerular hypertrophy with reduplication of the basement membrane and mesengial proliferation, as changes, which occur in sickle cell patients with increasing age.[12] These

changes lead to the hyperfiltration, which is observed in apparently normal patients and may render the GFR inefficient as a modality of assessing renal status in this group of patients. Proteinuria was observed in 20.3% of the patients, this low prevalence may be explained by the younger age of this patient population.

Clinical markers of disease severity in SCD, such as age and frequency of crisis per annum did not show any relationship with the occurrence of renal impairment. However, hyper-bilirubinaemia, which is an indicator of severe disease was obderved to be associated with a reduction in GFR. This may be explained by the observation that nephropathy was more attributable to the hemolytic, rather than the vaso-occlusive pathogenetic process in SCD. However, this may also imply that nephropathy in SCD does not necessarily occur in people with severe disease.

Important laboratory indices including Hb, platelet count and leucocyte count did not show any relationship with renal impairment as indicated by GFR. This is similar to the findings of the study by Guasch *et al*. and further suggests that nephropathy in sickle cell does not arise from a vaso-occlusive effect. Among the indicators of the increased hemolysis only the serum ALP and bilirubin showed an inverse relationship with the eGFR. However, further evaluation is required to ensure that this had not occurred secondary to the bony changes that occur with kidney disease. The serum bilirubin showed an inverse relationship with the GFR, however, Haymann *et al*. had observed a relationship with the occurrence of albuminuria and indicators of hemolysis. The Hb concentration however did reveal any trend as this would have further strengthened the argument that higher rates of haemolysis was a risk factor fro renal impairment. It is noteworthy that previous studies have shown the existence of a relationship between haemolysis and occurrence of albuminuria in 60% of patients, that a similar relationship may also exist with overt renal impairment.

The estimation of GFR using the MDRD seemed to be a more appropriate assessment of renal function, as it was both able to place a higher percentage of patients as normal as well as detect some of the patients to have other stages of CKD, unlike the CG. The eGFR_MDRD has also been shown to have a better correlation with proteinuria in sickle cell patients. Therefore, this study also indicates that the MDRD may be more appropriate in assessing renal impairment in the sickle cell population.

The presence of clinical and laboratory associations of renal impairment would undoubtedly assist the physician to identify likely victims of renal failure. This study, however, has revealed that the serum ALP and direct bilirubin have significant associations with renal impairment and that other clinical and laboratory indicators of disease severity may not be helpful. Further long term cohort studies may be needed to also evaluate the effect of genetic as well as environmental factors.

Conclusion

The eGFR_MDRD was suboptimal in 20.3% of the patients while a similar percentage (20.3%) also had proteinuria. The serum ALP, as well as direct bilirubin, showed significant inverse relationship with the eGFR_MDRD. Using the eGFR_MDRD seemed to be more appropriate in discerning renal impairment in homozygous S patients than the eGFR_CG. There was no significant relationship between the GFR and steady state clinical or laboratory parameters in sickle cell. Further studies will be required to rule the effect of environmental as genetic causative factors.

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