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β_2 Microglobulin as A Marker of Early Renal Damage In Patients With Sickle Cell Nephropathy

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Abstract
Introduction: *Sickle cell nephropathy is a cause of significant morbidity and mortality in patients with sickle cell disease. Traditional creatinine based assay for evaluating renal function has several limitations. Hence, the need for more sensitive markers such as β_2 microglobulin (β_2M).*
This study seeks to compare β_2M with creatinine as a marker of renal damage in patients with sickle cell disease.
Method: *This is a cross sectional study conducted at the University of Benin Teaching Hospital, Benin City, Nigeria. Serum creatinine and β_2M levels were determine using kinetic Jaffe method and β_2M ELISA assay (Quantikine kit) respectively in 83 sickle cell disease patients and 40 age and sex match controls. Estimated glomerular filtration rate (eGFR) was calculated using Cockcroft-Gault formular for eGFRCr estimation. Data was analyzed with SPSS version 16.*
Result: *β_2M in the SCD patients and controls were 25.0±3.9% versus 45.6±5.8% (p = 0.001), 17.4±7.5mg/dl versus 24.0±8.0mg/dl (0.001), 0.7±0.3mg/dl versus 0.9±0.3mg/dl (p = 0.003), 3.3±1.0 versus 2.3±0.7 (p = 0.001) respectively. The eGFR-Cr was higher in SCD patients than in controls (128.9±8.4ml/min/1.73m^2 versus 113.6±11.0ml/min/1.73m^2) but the difference was not statistically significant P = 0.316. β_2M has a positive correlation with urea, creatinine and a weak negative correlation with eGFRCr.*
Conclusion: *β_2M is superior to creatinine as a marker of sickle cell nephropathy.However, caution should be applied in patients with haematological malignancies and chronic inflammatory diseases.*
Keywords: *Sickle cell nephropathy, β_2M, Creatine, estimated GFRCr*

I. Introduction

Sickle cell disease is a genetic disease due to the inheritance of a sickle cell gene either in the homzygous state or in combination with another haemoglobinopathy such as thalassaemia and Hb C among others.[1] The disease is characterised by acute (crisis) and chronic complications involving multiple organ systems including the renal system. Renal complications of SCD are referred to sickle cell nephropathy (SCN). Renal complications contribute to a significant cause of morbidity and mortality in patients with SCD.[2]

The burden increases with age and it is estimated that 60% of SCD patients above 45years have microalbuminuria a marker of early renal damage; with 4 – 12% developing end stage renal disease[2,3].Platt et al[4] reported that almost 9% of SCD patients who died due to SCDcomplications manifested overt renal failure.This was identified as a major risk factor for early mortality in adult patients with SCD.

The underlying mechanisms include chronic hypoxia, medullary ischemia, and increased prostaglandin secretion that lead to glomerularhypertrophy, increased glomerular permeability, and proteinuria, which eventually result in chronic renal failure.[5]Furthermore, the hyperosmolar milieu of the medulla, favors hemoglobin S (HbS) polymerization andresults in increased blood viscosity within the medullarcapillaries, leading to loss of concentrating capacity, urinaryacidification, and decreased potassium excretion.[6] Thus, SCD results in both glomerular and tubular dysfunction which predates chronic renal failure.

The importance of the detection of early features and appropriate management of adverse changes in the renal system of SCD patients cannot be underestimated. Traditional methods of measuring renal impairment using creatinine has been widely critisized and adjudge to have serious limitations.[7] Its lack of sensivity to detecting early renal changes, subjectivity to spectral interference, dependence on gender and muscle mass necessitates the need for the use of other markers such as β_2-microglobulin a superior marker for the detection of renal impairment.

β_2-Microglobulin (β_2M), a low molecular weight protein measuring 11,800D. Ithas been identified as the light chain of the class I major histocompatibility antigens. Theyare found on the cell surface of all nucleated cells by which they are synthesized.[8]β_2Mis freely filtered in the glomerulus, totally reabsorbed and degraded in the renal tubules. Thus, it is a sensitive marker of the glomerular filtration capacity of the kidney. It is almost absent in urine. Urinary detection is also suggestive of tubular dysfuction.

The burden of SCN in Nigeria is enormous.[9, 10] However, there are paucity of studies on early markers of renal impairment such as β_2Min our environment hence this study was undertaken to evaluate the use β_2Min detecting renal changes in sickle cell patients in Benin City, Nigeria.

This study seeks to compare serum β_2Mto serum creatinine as markers of renal damage and to correlate β_2Mwith urea, creatinine, Cr based estimate glomerular filteration rate (eGFRCr)

II. Methodology

This is a cross sectional study conducted at the University of Benin Teaching Hospital Benin City, Nigeria. Eighty three Adult SCD patients in stable disease state (absence of major crises for a period of 3 months) were recruited consecutively from the Adult Haematology Outpatient Unit of the hospital. Their haemoglobin genotypes were determined by Hb electrophoreis in alkaline medium. Patients with established end stage renal disease on dialysis, those with haematological malignancies were excluded. Forty healthy volunteers were recruited as controls from the general population.

Particpants personal data were obtained using an interviewer administered questionaire. Their height and weight were measured with a stadiometer and weighing scales respectively. The weight and height measurements were used for the estimation of body mass index (BMI) with the formular:
BMI = Weight(Kg)/height(m)^2.

Thereafter 10 mls of venous blood was collected aseptically from the antecubital vein. Three milliliter (3ml) was dispensed into EDTA specimen bottle for packed cell volume estimation and 7 ml into a plain specimen container and allowed to clot. The clotted sample was centrifuged at 1500g for 15 minutes and serum was extracted and stored at -20^{o}C. The EDTA sample was used for estimation of packed cell volume.
The serum sample was used for determination of Creatinine and β_2M levels. Creatinine was determine usingkinetic Jaffe method while $\beta_2$microglobulin level was determine using ELISA method (Quantikine test kit). eGFR was determined using the Cockcroft-Gault formula:[11]
(140- Age in years) x Weight (kg)/72 x Creatinine(mg/dL) x 0.85(if Female).

The study was approved by the hospital ethical committee and all participants gave informed consent.
The data was analyzed with the statistical package for social sciences (SPSS) version 16. Student T test and chi square were used to compare parametric and non parametric variables between the SCD patients and controls. Pearson's correlation test was used for the correlation test. P value was set at 0.05.

III. Result

The demographic parameters of the SCD patients and controls are shown in table 1. There is no significant difference in the age and sex distribution between both groups. The mean body mass index is significantly lower in SCD patients than in the controls (p = 0.001).

Table 1: Age, body mass index and sex distribution of the study population

Variables	SCD (n=83) $\bar{X}$± SD	Controls (n=40) $\bar{X}$± SD	P value
Age (yrs)	27.2 ± 7.6	27.4 ± 9.5	0.768
BMI (Kg/m^2)	17.9±4.8	24.1±3.7	0.001
Sex	Freq (%)	Freq (%)	
Males	40 (48.2)	25 (62.5)	0.136
Females	43 (51.8)	15 (37.5)	

The mean packed cell volume (PCV), serum urea, creatinine and β_2M in the SCD patients and controls were 25.0±3.9% versus 45.6±5.8% (p = 0.001), 17.4±7.5mg/dl versus 24.0±8.0mg/dl (0.001), 0.7±0.3mg/dl versus 0.9±0.3mg/dl (p = 0.003), 3.3±1.0 versus 2.3±0.7 (p = 0.001) respectively. The eGFRCr was higher in SCD patients than in controls (128.9±8.4ml/min/1.73m^2 versus 113.6±11.0ml/min/1.73m^2) but the difference was not statistically significant P = 0.316 (Table 2).

Table 2: Haematological and biochemical parameters of the study population

Variables	SCD (n=83) $\bar{X}$± SD	Controls (n=40) $\bar{X}$± SD	P value
PCV (%)	25.0± 3.9	45.6 ± 5.8	<0.001
Urea (mg/dl)	17.4 ± 7.5	24.0 ± 8.0	<0.001
Creatinine (mg/dl)	0.7 ± 0.3	0.9 ± 0.3	0.003
β2 microglobin (mg/L)	3.3 ± 0.98	2.3 ± 0.7	<0.001
eGFRCr (ml/min/1.73m^2)	128.9 ± 8.4	113.6 ± 11.0	0.316

Table 3 shows the comparison of urea, Cr and β_2M between males and females within each of the study groups. In SCD the difference in means between Cr and β_2M in male and female patients were not significant

statistically (p = 0.353 and 0.114 respectively). However in the controls males have a significantly higher Cr than females (p = 0.001) but there was no significant difference in the mean β_2M level.

Table 3: Biochemical parameters based on sex distribution of the study population

Variables	SCD(n=83)			Controls (n=40)		
	M (n=40) $\bar{X}$± SD	**F (n=43) $\bar{X}$± SD**	**P value**	**M (n=25) $\bar{X}$± SD**	**F (n=15) $\bar{X}$± SD**	**P value**
Urea (mg/dl)	17.8±6.5	17.1±8.5	0.715	25.54±9.4	21.6±4.5	0.189
Creatinine (mg/dl)	0.7±0.3	0.7±0.3	0.934	1.1±0.3	0.7±0.1	0.001
β2 microglobin (mg/L)	3.5±1.1	3.1±0.9	0.114	2.3±0.7	2.3±0.6	0.815
eGFRCr (ml/min/1.73m^2)	136.4±15.0	122.5±8.0	0.854	109.3±17.0	120.3±9.2	0.637

Using eGFRCr, 21 (25.3%) of the SCD patients and 14 (35%) of the controls had subnormal renal function. The difference in not statistically significant (X2 = 1.247; p = 0.264). However using β_2M, 64 (77.1%) of the SCD patients had impaired renal function compared to 10 (25.0%) of the controls (X^2 = 30.58; p = 0.001) as in table 4.

Table 4: Comparison of the proportion of SCD patient and Controls with impaired renal function using eGFR and B_2M

Variables	SCD	Controls	X^2	P value
eGFR Cr				
<90 ml/min/1.73m^2	21 (25.3)	14 (35.0)	1.247	0.264
≥ 90 ml/min/1.73m^2	62 (74.7)	26 (65.0)		
β_2M				
≤2.7mg/dl	19 (22.9)	10 (25.0)	30.580	0.001
>2.7mg/dl	64 (77.1)	30 (75.0)		

Table 5 compares the proportion of subjects in each that has renal dysfunction using eGFR and that using β_2M. In the SCD group, using β_2M the proportion of renal dysfuntion detectable is significantly higher than with eGFR (X^2 = 48.58; p = 0.000). In the control (non SCD)group, no significant difference was found with use of B_2M and eGFRCr (X^2 = 0.952; p = 0.329).

Table 5: Comparison of B2M and eGFRCr as markers of Renal Dysfunction

Variables	SCD		X^2	P value
	eGFR Cr	**□$_2$M**		
Normal	62 (74.7)	19 (22.9)	44.580	0.000
Abnormal	21 (25.3)	64 (77.1)		
	Controls			
	eGFR Cr	**□$_2$M**		
Normal	26 (65.0)	30 (75.0)	0.952	0.329
Abnormal	14 (35.0)	10 (25.0)		

In the SCD patients, there were statistically significant positive correlations between β_2M and Urea(r = 0.548; p = 0.000) and between β_2M and Cr (0.533; p = 0.000). A negative correlation was established between β_2M and eGFR Cr but it was not statistically significant (r = -0.134; p = 0.222) (Table 6).

Table 6: Correlation between β_2M, Urea, Cr and eGFR Cr

		Urea	Creatinine	eGFR Cr
β_2M	R	0.548	0.533	-0.134
	P value	0.000	0.000	0.222

IV. Discussion

Sickle cell nephropathy (SCN) is a cause of significant morbidity and mortality in patients with sickle cell disease. The need for the use of a sensitive marker for detection of early changes in renal function cannot be overemphasized. Creatinine based assay has several limitations necessitating the need for the use of superior markers for the detection of renal injuries. β_2M is one of such markers capable of replacing Cr assay.

In this study, the mean Cr levels was significantly lower in SCD patients than in controls while eGFR Cr was higher in SCD patients than in controls though not statistically significant. The relatively lower Cr and higher eGFR Cr can be attributed to the lower body mass of the SCD subjectsand the associated glomerular hyperfilterationchanges.Hyperfiltration is one of the early parthological changes in SCN. However, the B_2M was significantly higher in the SCD group.

Sessoet al[12] in a comparative study on renal function in homozygous SS and heterozygous AS subjects reported a significantly increased β_2M in patients with SS. Similarly they observed a significantly lower serum creatinine and a significantly higher eGFR. In another related study, de Jong et al,[13] reported a significantly elevated β_2M in SCD compared to controls. Although measurement of Cr issimple and easily available, it is reported that almost 50% of patients withimpaired GFR have normal Cr[14]. This suggests that β_2M is a better marker of renal derangement thanthe eGFRCr in SCD.

The proportion of SCD patients with subnormal eGFRCr is 25.3% compared to 77.1% using β_2M estimation. This difference is statistically significant. Thus β_2M is superior to eGFRCr in detecting renal dysfunction. This is similar to the observations of Voskaridouet al[15] however, values observed in the index study were relatively higher than theirs.

Cr based eGFR is established to be dependent on age, body mass, sex and it is prone to interferences unlike β_2M. This study has shown that sex differences did not affect β_2M levels both in the SCD and control groups. Guido et al[16] demonstrated the age independence of β_2M making it a suitable marker for use in evaluating glomerular dysfunction across all age groups. β_2M has been observed to become elevated by the age of 20 years while significant observable deterioration in GFR becomes obvious by the age of 35 yrs in sickle cell patients.[17] Using $\beta_2M$as marker of renal dysfunctionin SCD patients, SCN will be detected earlier and appropriate intervention initiated compared to the use of the traditional eGFRCr estimation.

In conclusion, it is obvious that the use of β_2M to evaluate renal function in SCD is superior to creatinine and eGFRCr. However, caution should be exercised in interpreting resultsof patients with haematological malignancies and chronic inflammatory conditionssuch as connective tissue disease as its level will be elevated in these conditions.

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