

Original Research Article

CORRELATION OF MICROALBUMIN WITH BIOCHEMICAL AND ANTHROPOMETRIC PARAMETERS IN SICKLE CELL ANAEMIA PATIENTS IN SOUTHERN NIGERIA.

ABSTRACT

The renal manifestations of sickle cell anaemia (SCA) can range from haematuria and abnormal tubular function to massive proteinuria and end stage renal disease. This study was designed to screen for microalbuminuria in SCA patients and assess the coexistence of frequently documented biochemical changes and anthropometric parameters in sickle cell anaemia patients. This was achieved by determining the presence of microalbuminuria in these patients using Albumin Creatinine Ratio (ACR) in spot urine. A total of 68 asymptomatic sickle cell anaemia patients and 30 non sickle cell anaemia controls were used for the study. These patients were grouped into two: those with normal ACR and others with microalbuminuria using the $ACR \geq 3.0 \text{ mg/mmol}$ which approximates 24hr urinary albumin excretion in mg. Results obtained showed a mean significant increase ($P < 0.05$) in plasma urea in the SCA patients ($6.43 \pm 0.54 \text{ mmol/L}$) than in the control ($3.33 \pm 0.16 \text{ mmol/L}$). There was also a significant increase ($P < 0.05$) in ACR in the SCA patients ($4.5 \pm 0.24 \text{ mg/mmol}$) than in the control subjects ($2.19 \pm 0.10 \text{ mg/mmol}$). Packed cell volume (PCV) was significantly higher ($P < 0.05$) in the control subjects (40.65 ± 0.62) than in the SCA patients (23.48 ± 0.94). There was a significant positive correlation between ACR and plasma urea in SCA patients with nephropathy ($n = 52$; $r = 0.58$, $P < 0.05$) and in those without nephropathy ($n = 16$, $r = 0.36$, $P < 0.05$). The relationship between ACR and PCV was a significant negative correlation in SCA patients with nephropathy ($n = 52$; $r = -0.39$, $P < 0.05$).

27 0.05). Our analysis also indicated that there was a significantly higher waist hip ratio (WHR)
28 and systolic blood pressure in sickle cell anaemia patients (0.89 ± 0.01 , 120.39 ± 1.56 mmHg; P
29 < 0.05) than in the control (0.86 ± 0.01 , 111.62 ± 1.97). Body mass index (BMI) was
30 significantly lower in the SCA patients ($P < 0.05$). The study also showed that the incidence of
31 nephropathy amongst sickle cell anaemia patients in Southern Nigeria is 76.5%.
32 Microalbuminuria is a common finding in SCA patients and monitoring of ACR will be
33 important in detecting early onset of sickle cell nephropathy

34 KEY WORDS: Sickle cell anaemia, Sickle cell nephropathy, Albumin creatinine ratio, Plasma
35 creatinine, Waist- hip ratio, Body mass index.

36 INTRODUCTION

37 Sickle cell anaemia is a genetic disorder characterized by red blood cells that assume an
38 abnormal rigid sickle shape [1]. The major consequences of sickle cell anaemia are vascular
39 occlusion by sickled cells and anaemia because of red blood cell destruction. Sickle cell
40 anaemia (SCA) is a heterogenous disorder with clinical manifestations including chronic
41 haemolysis, an increased susceptibility to infections and vaso-occlusive complications as
42 well as extensive organ damage reducing both their quality of life and their life expectancy
43 [2].

44 The survival of children with SCA into adulthood has increased the probability of developing
45 some degree of renal insufficiency [3]. Every year 150,000 babies are born homozygous for
46 SCA in Nigeria which is by far the most populous country in the West African sub-region. The
47 frequency of the sickle cell trait (healthy carriers) is 5% of the world population and 15% -
48 30% in West African countries such as Ghana and Nigeria [4]. The prevalence of chronic
49 renal insufficiency in SCA from a previous study was 4.6% [5].

50 The sickling process may cause haematuria, renal papillary necrosis and urinary
51 concentrating defect which may progress to sickle cell nephropathy [6]. Microalbuminuria,
52 also known as albumin excretion rate >30mg/24hrs or ACR >30mg/g (>3.0mg/mmol), is used
53 as a marker of renal damage and is used to define chronic kidney disease [7].

54 Sickle cell nephropathy is now a well characterized entity with specific manifestations
55 riskfactors and prognosis [8]. Progressive severe anaemia occurring in these patients
56 secondary to ineffective erythropoiesis is a major risk factor in renal disease [9]. This study
57 intends to screen for microalbuminuria in SCA patients and also assess the coexistence of
58 frequently documented biochemical changes in sickle cell nephropathy.

59 PATIENTS AND METHODS

60 This study was carried out in the Department of Biochemistry University of Benin and the
61 sickle cell Centre of the Ministry of Health Benin City, Edo State, Nigeria. A written informed
62 consent was obtained from each volunteer. Ethical clearance was obtained from the
63 research and ethical committee of the Ministry of Health Benin City, Edo State. SCA patients
64 were those between the ages of 18 and 60 years, attending the sickle cell clinic while the
65 control subjects were non-SCA patients of the same age limit with normal ACR and renal
66 function attending the out-patient clinic. Sample size was calculated from the prevalence of
67 chronic renal insufficiency in SCA using the Cochrane formula [10]. Data was obtained from
68 all consenting volunteers (68 SCA patients and 30 non SCA control subjects), using a
69 structured questionnaire to obtain information such as age, sex, religion, occupation, family
70 history of SCA, and educational status. Patients who had nephropathy were those patients
71 whose ACR was $\geq 3.0\text{mg/mmol}$.

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73 **SAMPLE COLLECTION**

74 In the morning (at about 9.00AM) of their appointment at the Sick Cell Centre, 5.0ml of
75 blood was obtained from cubital fossa using a 5.0ml syringe and 3.0ml of blood was
76 dispensed into lithium heparin specimen bottle for plasma urea and creatinine
77 estimation while 2.0 ml of blood was dispensed into ethylenediaminetetraacetic acid (EDTA)
78 bottle for immediate estimation of PCV. Random spot urine specimen was also collected into
79 a sterile universal bottle for urine albumin and creatinine estimation. Since this was more
80 convenient for the patients than a timed (24hrs or overnight) urine collection. The blood
81 specimens in the lithium heparin bottles were immediately centrifuged at 3000g for 5
82 minutes to separate plasma from the cellular components. The plasma was stored at 20°C
83 until analysis was carried out for the different assays while urine was assayed immediately
84 for albumin and creatinine.

85 **BIOCHEMICAL ANALYSIS**

86 Plasma urea was estimated by the urease – Berthelot method as outlined by Weatherburn
87 [11], plasma creatinine by the modified Jaffe's method as outlined by Spierto et al [12] and
88 urinary albumin by the Lowry method [13]. Packed cell volume was estimated using the
89 microhaematocrit method [14].

90 **DATA ANALYSIS**

91 Data analysis was done using the statistical package for social science (SPSS) version 16.0.
92 Categorical data were expressed as frequencies (percentages) and compared using a
93 Pearson Chi-square test. Continuous data were presented as mean $\pm$ standard error of mean
94 (SEM). Comparison of two independent variables was done using an independent t-test

95 while 3 groups were compared using one way analysis of variance (ANOVA). Pearson's
96 correlation was used to determine relationship between ACR and selected variables. The
97 confidence limit was 95% and P-value was considered significant at a value less than 0.05.
98 Also bar chart was used to express occurrence of distribution and drawn using Microsoft
99 Excel, 2007.

100 RESULTS

101 Table 1 showed the anthropometric parameters of the SCA patients and control subjects.
102 The SCA patients had significantly lower body mass index (BMI), higher waist hip ratio (WHR)
103 and systolic blood pressure (SBP) ($p < 0.05$), while the diastolic blood pressure was non-
104 significantly ($p > 0.05$) elevated. There was also a significantly lower packed cell volume
105 (PCV) in the SCA patients ($p < 0.05$). The biochemical parameters in table 2 showed a
106 significantly higher albumin creatinine ratio (ACR) ($p < 0.05$) and significantly elevated plasma
107 urea and creatinine level in SCA patients than in the control subjects ($p < 0.05$). In table 3,
108 ACR, plasma urea and creatinine were significantly elevated ($p < 0.05$) in the SCA patients
109 with nephropathy than in those without nephropathy and control subjects. The systolic and
110 diastolic blood pressure were found to be associated with microalbuminuria ($P < 0.05$) while
111 WHR and BMI had no significant association with ACR ($P > 0.05$). This study showed a
112 significant association between ACR and plasma urea and between ACR and PCV ($p < 0.05$)
113 while there was a non-significant association between ACR and plasma creatinine ($p >$
114 0.05) (table 4). Multivariate linear regression analysis done between PCV, plasma urea and
115 ACR were not statistically significant ($p > 0.05$).

116 Table 5 showed a significant positive correlation between plasma urea and ACR ($p < 0.05$) and
117 a negative non-significant correlation between ACR and PCV ($p > 0.05$). Fig 1 showed the

frequency of age distribution among the SCA patients with majority of the patients being in the age range of 18 – 27 years. Figure 2 displays the relationship between ACR and PCV in the SCA patients.

TABLE I

Characteristics of subjects and controls

Anthropometric parameter	Control subjects n = 30	SCA patients n = 68	P-value
Gender (male/female)	15/15	26/42	0.49
Age (years)	28.35 ± 0.42	30.12 ± 1.4	0.08
BMI (kg/m ²)	24.63 ± 0.68	20.85 ± 0.38	< 0.001
WHR	0.86 ± 0.01	0.89 ± 0.01	0.04
SBP (mmHg)	111.62 ± 1.97	120.39 ± 1.56	0.04
PCV	40.65 ± 0.62	23.48 ± 0.94	< 0.001
DBP	75.00 ± 1.64	82.36 ± 1.62	0.14

BMI = Body Mass Index

WHR = Waist Hip Ratio

SBP = Systolic Blood Pressure

PCV = Packed Cell Volume

DBP = Diastolic Blood Pressure

TABLE 2

Biochemical parameters of SCA patients

Biochemical parameter	Control subjects n = 30	SCA patients n = 68	P-value
Plasma urea (mmol/L)	3.33 ± 0.16	6.43 ± 0.54	<0.001
Plasma creatinine (μmol/L)	73.98 ± 2.95	86.70 ± 6.08	<0.001
ACR (mg/mmol)	2.19 ± 0.10	4.50 ± 0.24	<0.001

ACR= Albumin-creatinine ratio

SCA = Sickle cell anaemia.

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137 **TABLE 3**138 **Biochemical parameters of SCA patients with and without nephropathy**

Biochemical parameter	Control subjects n = 30	SCA patients (n = 68)		P-value
		With nephropathy (n = 52; ACR $\geq$ 3.0mg/mmol)	Without nephropathy (n = 16; ACR < 3.0 mg/mmol)	
Plasma urea (mmol/L)	3.33 $\pm$ 0.16	6.92 $\pm$ 0.67	4.85 $\pm$ 0.66	< 0.001
Plasma creatinine (μ mol/L)	73.98 $\pm$ 2.95	87.61 $\pm$ 6.09	83.73 $\pm$ 8.68	< 0.001
ACR (mg/mmol)	2.19 $\pm$ 0.10	5.14 $\pm$ 0.25	2.45 $\pm$ 0.85	< 0.001
BMI	24.63 $\pm$ 0.68	20.84 $\pm$ 3.21	20.97 $\pm$ 6.64	> 0.05
WHR	0.86 $\pm$ 0.01	0.88 $\pm$ 0.08	0.91 $\pm$ 0.04	> 0.05
SBP	111.62 $\pm$ 1.97	119.04 $\pm$ 17.29	105.62 $\pm$ 7.19	< 0.05
DBP	75.0 $\pm$ 1.64	82.69 $\pm$ 1.34	70.63 $\pm$ 2.49	< 0.05

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140 ACR = Albumin Creatinine Ratio

141 SCA = Sickle Cell Anaemia

142 BMI = Body mass index

143 WHR = Waist-Hip ratio

144 SBP = Systolic blood pressure

145 DBP = Diastolic blood pressure

146 **TABLE 4**147 **Correlation between ACR, plasma urea and creatinine in SCA patients with nephropathy (n = 52).**

Biochemical parameters	r-value	P – value
Plasma urea	0.58	0.04
Plasma creatinine	0.35	0.19
PCV	-0.39	0.02

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149 ACR = Albumin Creatinine Ratio

150 PCV = Packed Cell Volume

151 SCA = Sickle Cell Anaemia

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155 **TABLE 5**

156 **Correlation between ACR and plasma urea and creatinine in SCA patients without nephropathy (n**
157 **= 21).**

Biochemical parameter	r – value	P –value
Plasma urea	0.36	0.04
Plasma creatinine	0.20	0.35
PCV	0.20	0.42

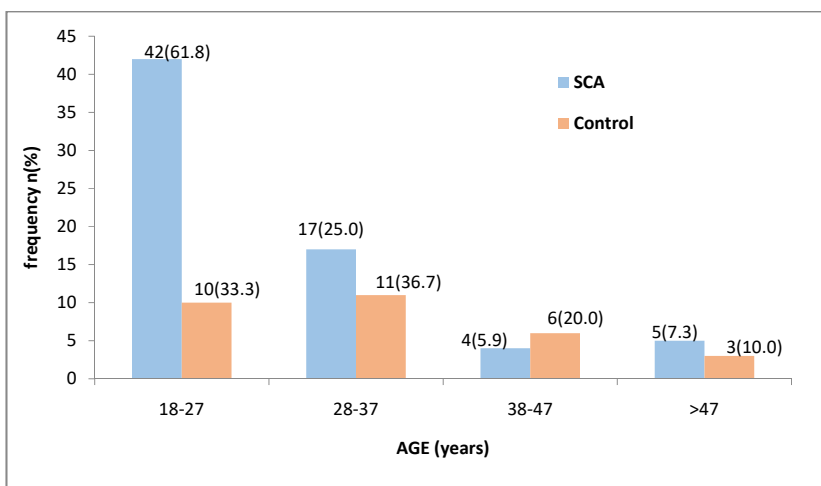
158 ACR = Albumin Creatinine Ratio

159 PCV = Packed Cell Volume

160 SCA = Sickle Cell Anaemia

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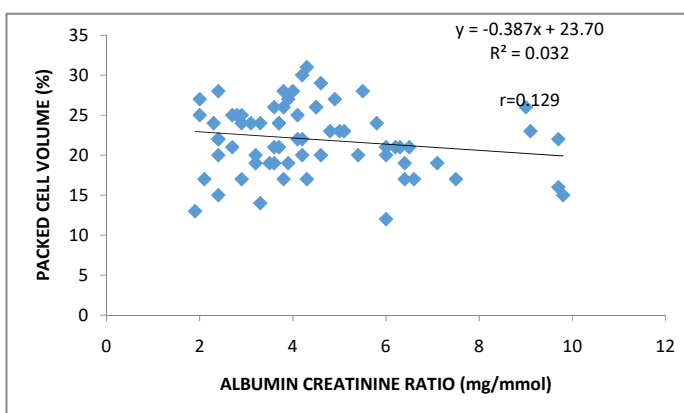
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164 Figure 1: Age distribution of Sickle cell anaemia (n = 68) and non-sickle cell anaemia (control)

165 respondents (n = 30). Values in parenthesis represent percentage distribution.



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168 **Figure 2: Relationship between Albumin Creatinine Ratio and Packed Cell**

169 **Volume of sickle cell anaemia respondents (n=68)**

170 **Discussion**

171 In Nigeria which is by far the most populous country in sub Saharan Africa, the prevalence of
 172 sickle cell **anaemia** (SCA) is about 2% [4]. Patients with SCA can develop life threatening
 173 complications as well as extensive organ damage reducing their quality of life and life
 174 expectancy [2].Sickle cell nephropathy (SCN) is a major mortality risk factor in**SCA** patients
 175 and it is associated with proteinuria that promotes progressive decline in renal function
 176 [15].

177 Microalbuminuria also called incipient nephropathy refers to albumin excretion rate of
 178 **>30mg/day (>20µg/ min) or ACR ≥ 3.0mg/mmol** in spot urine [16]. Among the SCA patients
 179 in this study, **76.5%** had microalbuminuria (ACR **≥3.0mg/mmol**). This is much higher than
 180 what Van Eps and de Jong obtained in their study where they found proteinuria in 30% of
 181 their patients during long term follow up [17]. Also, Bolarinwaet. al., in their study had 50%
 182 of their patients with albuminuria[18]. These differences could be attributed to the
 183 difference in availability of health care facilities in the environment of study. A secondary
 184 health facility was used in this study.The mean systolic and diastolic blood pressure in
 185 albuminuric subjects was significantly higher than in the non-albuminuric subjects which is

186 consistent with the increased cardiovascular risk of proteinuria; as supported by Bolarinwa
187 et. al, who studied renal disease in adult Nigerians with SCA [18].

188 The mean ACR in SCA patients with microalbuminuria was $5.14 \pm 0.25 \text{ mg/mmol}$
189 which was much higher than in those without microalbuminuria ($2.45 \pm 0.85 \text{ mg/mmol}$). This
190 corroborates earlier reports by Bolarinwa et. al., in their study in which they found a mean
191 ACR of $97 \pm 13.2 \mu\text{g/mg}$ (10.97 mg/mmol) in patients with microalbuminuria and $10.3 \pm$
192 $6.8 \mu\text{g/mg}$ (1.17 mg/mmol) in those without microalbuminuria [18].

193 The SCA patients had normal plasma urea ($6.43 \pm 0.5 \text{ mmol/l}$) and creatinine ($86.70 \pm$
194 $6.03 \mu\text{mol/l}$) though higher than in the control subjects. This finding is similar to that
195 obtained by Bolarinwa et. al. [18]. This implies that using plasma urea and creatinine as
196 indices of renal impairment in SCA patients is unreliable..

197 The finding that PCV or haematocrit level correlated negatively with ACR indicates that low
198 PCV is an index of disease severity which is in line with the finding of Faulkner et. al., where
199 they indicated that the lower the haematocrit level, the higher the urinary albumin
200 excretion rate [19]. Powar et. al. also reported progressive severe anaemia as a risk factor for
201 renal disease in SCA patients [20]. Faulkner et. al. also postulated that renal injury could
202 result from progressive medullary ischaemia due to severe progressive anaemia which may
203 lead to continuous activation of the prostaglandin pathway to produce vasodilator
204 prostaglandins as a compensatory mechanism to help maintain renal blood flow and
205 glomerular filtration rate (GFR) [19].

206 **Conclusion:** This study revealed that albuminuria is a common finding amongst SCA
207 patients. Albuminuria indicates the presence and progression of renal insufficiency.
208 Therefore regular monitoring of urine albumin is advocated in SCA patients to delay
209 progression of renal dysfunction to end stage renal disease.

210 **Consent:** All the participants were properly briefed about the study and an informed
211 consent was obtained from them before venous blood and urine was collected.

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