

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 a 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 ($6.34 \pm 0.54 \text{ mmol/L}$) 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 anemia is a genetic disorder characterized by red blood cells that assume an
38 abnormal rigid sickle shape [1]. The major consequences of sickle cell anemia are vascular
39 occlusion by sickled cells and anemia because of red blood cell destruction. Sickle cell
40 anemia (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 $>30\text{mg}/24\text{hrs}$ or $\text{ACR} >30\text{mg}/\text{g}$ ($>3.0\text{mg}/\text{mmol}$), 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.

71

72 **SAMPLE COLLECTION**

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

84 **BIOCHEMICAL ANALYSIS**

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

89 **DATA ANALYSIS**

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

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

99 RESULTS

100 Table 1 showed the anthropometric parameters of the SCA patients and control subjects.
101 The SCA patients had significantly lower body mass index (BMI), higher waist hip ratio (WHR)
102 and systolic blood pressure (SBP) ($P < 0.05$), while the diastolic blood pressure was non-
103 significantly ($P > 0.05$) elevated. There was also a significantly lower packed cell volume (PCV)
104 in the SCA patients ($P < 0.05$). The biochemical parameters in table 2 showed a
105 significantly ratio (ACR) ($P < 0.05$) and a non-significantly elevated plasma creatinine in SCA
106 patients than in the control subjects ($P > 0.05$). elevated plasma urea and albumin creatinine.
107 In table 3, ACR, plasma urea and creatinine were significantly elevated ($P < 0.05$) in the SCA
108 patients with nephropathy than in those without nephropathy and control subjects. This
109 study showed a significant positive correlation between ACR and plasma urea and a
110 significant negative correlation between ACR and PCV ($P < 0.05$) while there was a non-
111 significant positive correlation between ACR and plasma creatinine ($P > 0.05$) (table 4).
112 Table 5 showed a non-significant positive correlation between ACR and urea and creatinine ($P > 0.05$)
113 and a negative correlation between ACR and PCV which was also not significant ($P > 0.05$).
114 Fig 1 showed the frequency of age distribution among the SCA patients with majority
115 of the patients being in the age range of 18 – 27 years. Figure 2 displays the relationship
116 between ACR and PCV in the SCA patients.

117 **TABLE I**

118 **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

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120 BMI = Body Mass Index

121 WHR = Waist Hip Ratio

122 SBP = Systolic Blood Pressure

123 PCV = Packed Cell Volume

124 DBP = Diastolic Blood Pressure

125 **TABLE 2**

126 **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

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128 **ACR= Albumin-creatinine ratio**

129 **SCA = Sickle cell anaemia.**

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133 **TABLE 3**

134 **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	< 0.01
ACR (mg/mmol)	2.19 $\pm$ 0.10	5.14 $\pm$ 0.25	2.45 $\pm$ 0.85	< 0.001

135

136 ACR = Albumin Creatinine Ratio

137 SCA = Sickle Cell Anaemia

138 **TABLE 4**

139 **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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141 ACR = Albumin Creatinine Ratio

142 PCV = Packed Cell Volume

143 SCA = Sickle Cell Anaemia

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152 **TABLE 5**

153 **Correlation between ACR and plasma urea and creatinine in SCA patients without nephropathy (n**
 154 **= 21).**

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

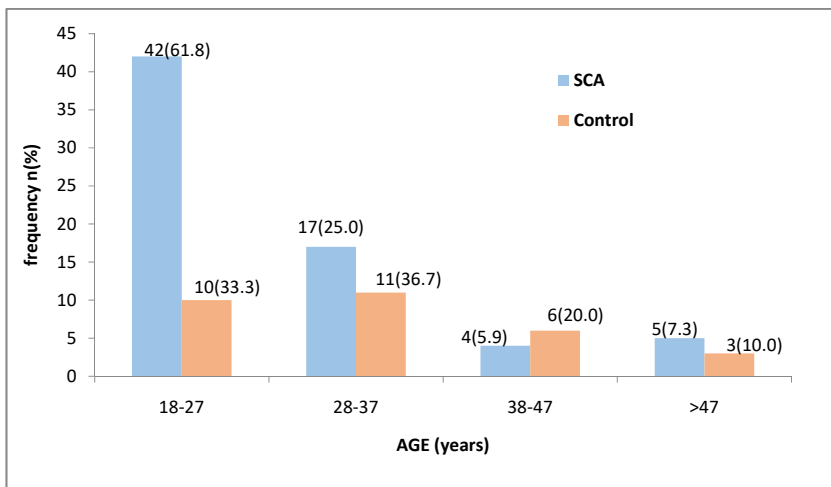
155 ACR = Albumin Creatinine Ratio

156 PCV = Packed Cell Volume

157 SCA = Sickie Cell Anaemia

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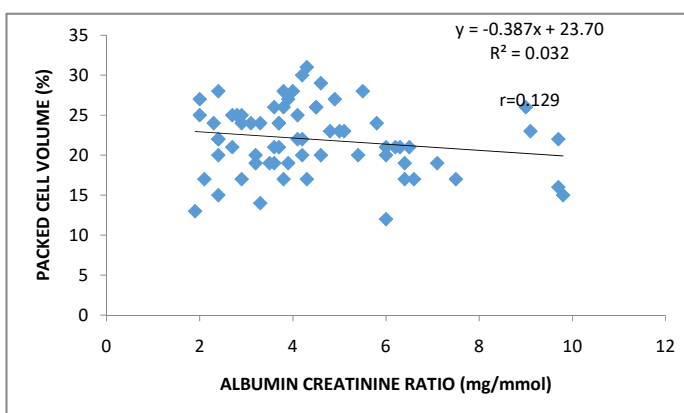
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161 Figure 1: Age distribution of Sickie cell anaemia (n = 68) and non-sickie cell anaemia (control)

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



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

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

167 Discussion

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

174 Microalbuminuria also called incipient nephropathy refers to albumin excretion rate of
 175 **>30mg/day (>20µg/ min) or ACR ≥ 3.0mg/mmol** in a spot urine [16]. Among the SCA patients
 176 in this study, **76.5%** had microalbuminuria (ACR **≥3.0mg/mmol**). This is much higher than
 177 what Van Eps and de Jong obtained in their study where they found proteinuria in 30% of
 178 their patients during long term follow up [17]. Also, Bolarinwaet. al., in their study had 50%
 179 of their patients with albuminuria[18]. These differences could be attributed to the
 180 difference in availability of health care facilities in the environment of study. A secondary
 181 health facility was used in this study.

182 The mean ACR in SCA patients with microalbuminuria was $5.14 \pm 0.25 \text{ mg/mmol}$
183 which was much higher than in those without microalbuminuria ($2.45 \pm 0.85 \text{ mg/mmol}$). This
184 corroborates earlier reports by Bolarinwa et. al., in their study in which they found a mean
185 ACR of $97 \pm 13.2 \mu\text{g/mg}$ (10.97 mg/mmol) in patients with microalbuminuria and $10.3 \pm$
186 $6.8 \mu\text{g/mg}$ (1.17 mg/mmol) in those without microalbuminuria [18].

187 The SCA patients had normal plasma urea ($6.43 \pm 0.5 \text{ mmol/l}$) and creatinine ($86.70 \pm$
188 $6.03 \mu\text{mol/l}$) though higher than in the control subjects. This finding is similar to that
189 obtained by Bolarinwa et. al. [18]. This implies that using plasma urea and creatinine as
190 indices of renal impairment in SCA patients is unreliable..

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

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

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

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