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Correlation of sialic acid and anthropometric variables in sickle cell anaemia patients

EO Onovughakpo Sakpa[1], EC Oyeneke[2], E Ayinbuomwan[3*], JC Anionye[4]
[1, 3] Department of Chemical Pathology, University of Benin, Benin City, Edo, Nigeria
[2] Department of Biochemistry, University of Benin, Benin City, Edo, Nigeria
[4] Department of Medical Biochemistry, University of Benin, Benin City, Edo, Nigeria

Abstract
Sickle cell anaemia (SCA) is characterized by sickled red blood cells which cause micro-infarct formation, ischaemia, decreased medullary blood flow and papillary necrosis. Sickle red blood cells have been reported to have a lower than normal membrane sialic acid content resulting in an abnormal interaction between sickle red blood cells and the microvascular endothelium. This deficiency is possibly reflected in a commensurate removal of the compound from the circulation. This study was designed to assess serum sialic acid (SSA) in SCA patients and its relationship with some anthropometric variables in SCA patients. This was achieved by determining the level of sialic acid in the serum of these patients. A total of 68 asymptomatic SCA patients and 30 non sickle cell anaemia controls were used for the study.
Results obtained showed that the SCA patients had a significantly ($P < 0.05$) lower BMI ($20.85 \pm 0.68 kg/m^2$), higher WHR (0.89 ± 0.01) and lower sBP (111.6 ± 1.97mmHg) than the control subjects with mean BMI of 24.63 ± 0.68 kg/m^2, WHR 0.86 ± 0.01, and SBP 116.0 ± 1.86 mmHg. There was a significantly ($P < 0.05$) elevated plasma urea (6.43 ± 0.54mmol/L) and the serum sialic acid (1.88mmol/L) was significantly ($P < 0.05$) lower in the SCA patients than in the control with a plasma urea of 3.33 ± 0.16mmol/L, and SSA of 1.93 ± 0.67mmol/L. SSA was also found to be significantly ($P < 0.05$) higher in females (1.93 ± 0.09mmol/L) than in males (1.80 ± 0.09mmol/L).
There was a negative correlation between SSA and dBP ($r = - 0.03$, $P > 0.05$) while a positive correlation was found between SSA and sBP ($r = 0.05$, $P > 0.05$), BMI ($r = 0.04$, $P > 0.05$), WHR ($r = 0.11$, $P > 0.05$) and plasma creatinine ($r = 0.17$, $P > 0.05$). A significant positive correlation was found between SSA and plasma urea ($r = 0.27$, $P < 0.05$) in the SCA patients. Serum sialic acid was lower in SCA patients and it is clinically correlated with WHR, BMI, and blood pressure which are prognostic factors for cardiovascular disease. SSA is also clinically correlated with plasma urea and creatinine. SSA can therefore be used as a predictor of cardiovascular and renal complications in SCA.

Keywords: sickle cell anaema (SCA), Serum sialic acid (SSA), plasma urea and creatinine, body mass indwx (BMI), waist to hip ratio (WHR)

1. Introduction
Sickle cell anaemia is characterized by sickled red blood cells which cause micro-infarct formation, ischaemia, decreased medullary blood flow and papillary necrosis [1]. SCA is common among people from Sub-Saharan Africa, India, Saudi Arabia and Mediterranean countries 2. The carrier rate of SCA ranges between 10% - 40% across equatorial Africa and in West African countries such as Ghana and Nigeria, the frequency of the trait is 15% - 30% 2. In Nigeria which is the most populous country in the sub region, 24% of the population are carriers of the mutant gene and the prevalence of SCA is about 2% [2].
Sialic acid (n-acetylneuraminic acid, NANA) is a generic term used for the N and O substituted derivative of neuraminic acid a monosaccharide with a nine carbon backbone [3]. It is a major component of membrane glycoprotein which is important in the maintenance of cellular integrity [4]. Elevated levels may indicate excessive cell damage especially of vascular tissue. [5] This leads to ischaemia which occur mostly in the small blood vessels of the retina, kidney, heart and brain [5]. Sickle red blood cells have been reported to have a lower than normal membrane sialic acid (NANA) content [6]. Since NANA is the major determinant of negative surface charge on red blood cells [7], such a deficiency would provide a potential mechanism for an abnormal interaction between sickle red blood cells and the microvascular endothelium [8]. Ekeke and Ibeh also observed an age dependent decrease in sialic acid in the serum of sicklers, possibly implying that the loss of sialic acid in erythrocyte membrane is reflected in a commensurate removal of the compound from the circulation [9].
The non-specificity of serum sialic acid (SSA) limits its clinical usefulness. Nevertheless, when combined with other indices, SSA concentrations are helpful in disease screening and follow-up as well as in monitoring treatment [10]. This study was done to correlate serum sialic acid levels with some anthropometric variables in SCA patients in Southern Nigeria.

Materials and methods
This study was carried out in the Department of Biochemistry University of Benin and the Sickle cell Centre of the Ministry of Health Benin City, Edo State, Nigeria. A written informed consent was obtained from each volunteer. Ethical clearance was obtained from the research and ethical committee of the Ministry of Health Benin City, Edo State. SCA patients were

those between the ages of 18 and 60 years attending the sickle cell clinic while the control subjects were non-SCA subjects of the same age limit.

Sample Collection : In the morning (at about 9:00am) of their appointment at the Sickle Cell Centre, 7.0ml of venous blood was obtained from the cubital fossa using 10.0ml syringe. About 4.0mls of blood was dispensed into lithium heparin bottle for plasma urea and creatinine estimation while 3.0mls of the sample was dispensed into a plain bottle and allowed to clot for sialic acid estimation. Samples were centrifuged at 3000g. Plasma / serum was then harvested and stored at 2 - 8oC and analyzed within 48hrs for plasma urea and creatinine and sialic acid.

Biochemical Analysis

Plasma urea was estimated by urease – Berthelot method as outlined by Weatherburn [11], plasma creatinine by the modified Jaffe's method as outlined by Spierto *et al.* [12] and serum sialic acid was analysed using the Standard Ehrlich method [13].

Data analysis

Data analysis was done using the statistical package for social science (SPSS) version 16.0. Continuous data were presented as mean ± standard error of mean (SEM). Comparison of two independent variables was done using regression coefficient correlation and analysis of variance (ANOVA) was applied for comparison between different groups. The confidence limit was 95% and the P – value was considered significant at a value less than 0.05.

Results

Table 1 showed the anthropometric parameters of the control (non- sickle cell anaemia) subjects and the SCA patients. The SCA patients had significantly (P< 0.05) lower body mass index (BMI), higher waist hip ratio (WHR) and lower systolic (SBP) than the control subjects.

The biochemical variables in table 2 showed a significantly (P < 0.05) elevated plasma urea and a non-significant (P > 0.05) increase in plasma creatinine in the SCA patients. The serum sialic acid concentration was significantly (P< 0.05) lower in the SCA patients when compared to the control subjects.

Table 3 shows gender considerations of some biochemical variables in control subjects and SCA patients. In the SCA patients, the plasma creatinine is numerically, though not significantly elevated (P>0.05) in males (95.39 ± 9.25µmol/L) than in the females (81.31 ± 7.91µmol/L). There was also no significant difference in mean plasma urea between male and female SCA patients. However, SSA was significantly higher (P < 0.05) in females (1.93 ± 0.09mmol/L) than in males (1.80 ± 0.09mmol/L) in the SCA patients.

Figures 1, 2 and 3 show the relationship between SSA and systolic blood pressure, diastolic blood pressure and BMI in SCA patients. There was no significant correlation between SSA and systolic blood pressure (r = 0.05, P = 0.73; Fig 1), diastolic blood pressure (r = - 0.03, P = 0.81; Fig 2) and BMI (r = 0.04, P = 0.73; Fig 3). However there was a positive correlation between SSA and WHR (r = 0.11, P = 0.36; Fig 4). Figures 5 and 6 show the relationship between serum sialic acid and plasma urea and creatinine. There were positive correlations between SSA and plasma urea (r = 0.27, P = 0.03; Fig 5) and creatinine (r = 0.17, P = 0.16; Fig 6).

Table 1: Age, BMI, WHR and blood pressure measurements in control (non sickle cell anaemia) subjects and sickle cell anaemia patients.

Clinical Parameters	Male (n)	Female (n)	Age (Years)	BMI (Kg/m^2)	WHR	Dpb (mmHg)	sBP (mmHg)
Control (non SCA) subjects	15	15	33.12±1.14	24.63±0.6$_{8}$^a	0.86 ±0.01^a	75.00±1.64^a	116.0±1.56^a
SCA patients	26	42	28.35±0.42	20.85±0.3$_{8}$^b	0.89 ± 0. 01^a	72.35±0.01^a	111.62 ±1.97^b

Values in the same column with different alphabets differ significantly (p<0.05)

SCA- Sickle cell anaemia, BMI- Body mass index, WHR- Waist hip ratio, dBp- Diastolic blood pressure, sBp- Systolic blood pressure

Table 2: Plasma urea and creatinine, and SSA in control (non SCA) subjects and SCA patients.

Subjects/ Patients	Plasma Urea (mmol/L)	Plasma Creatinine (µmol/L)	SSA (mmol/L)
Control (non SCA) (n=30)	3.33 ± 0.16^a	73.98 ± 2.95^a	1.93 ± 0.67^a
SCA (n=68)	6.43 ±0.54^b	86.70 ± 6.08^a	1.88 ± 0.96^b

Values in the same column with different alphabets differ significantly (p<0.05)

SCA- Sickle cell anaemia, ACR- Albumin creatinine ratio, SSA- Serum sialic acid

Table 3: Plasma urea, creatinine, urine albumin ACR and SSA; Gender consideration in control (non SCA) subjects and SCA patients.

Biochemical Parameter	Control (non SCA; n = 30)			SCA (n = 68)		
	(m = 15)	(F = 15)	Total	(m = 21)	(F = 47)	Total
Plasma urea (mmol/L)	3.64 ± 0.22^{a}	3.01 ± 0.22^{a}	3.33 ± 0.16^{c}	7.10 ± 0.96^{a}	6.02 ± 0.65^{a}	6.43 ± 0.54^{d}
Plasma creatinine (µmol/L)	72.34 ± 4.73^{a}	75.62 ± 3.66^{a}	73.98 ± 2.95^{c}	95.39 ± 9.15^{a}	81.31 ± 7.91^{a}	86.70 ± 6.03^{c}
SSA (mmol/L)	2.03 ± 0.09	1.82 ± 0.09	1.93 ± 0.67^{c}	1.80±0.09^{a}	1.93±0.09^{b}	1.88 ± 0.96^{c}

Values in the same row with different alphabets differ significantly (P< 0.05)

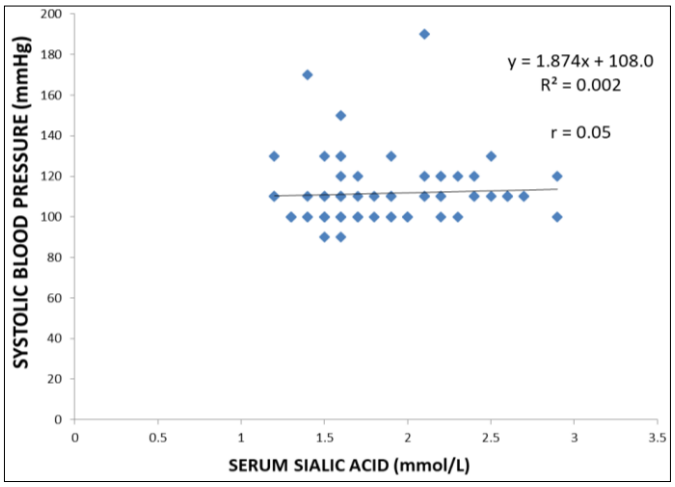

Fig 1: Relationship between serum sialic acid and systolic blood pressure in sickle cell anaemia patients (n=68)

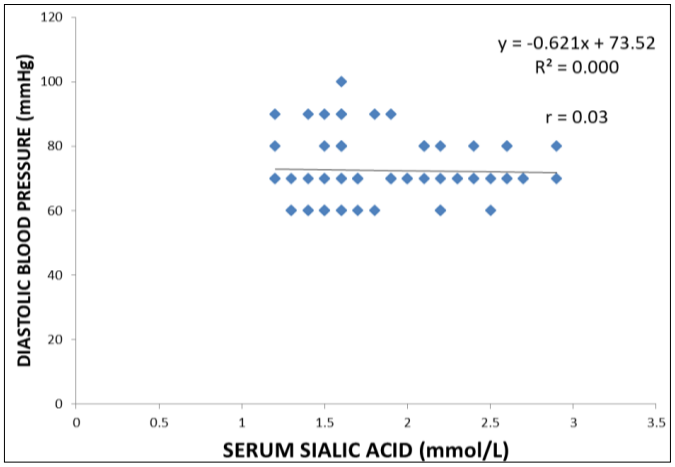

Fig 2: Relationship between serum sialic acid and diastolic blood pressure in sickle cell anaemia patients (n=68)

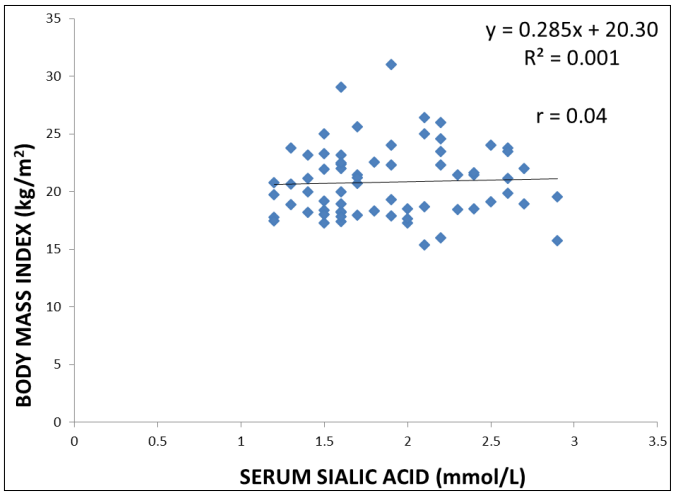

Fig 3: Relationship between serum sialic acid and BMI in sickle cell anaemia patients (n=68)

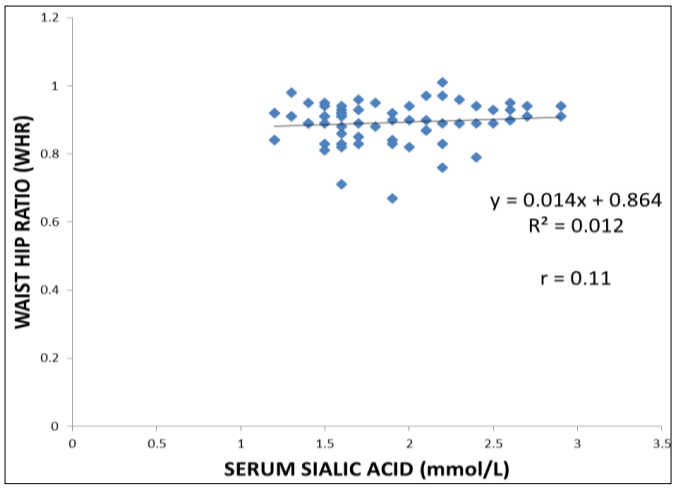

Fig 4: Relationship between serum sialic acid and waist hip ratio in sickle cell anaemia patients (n=68)

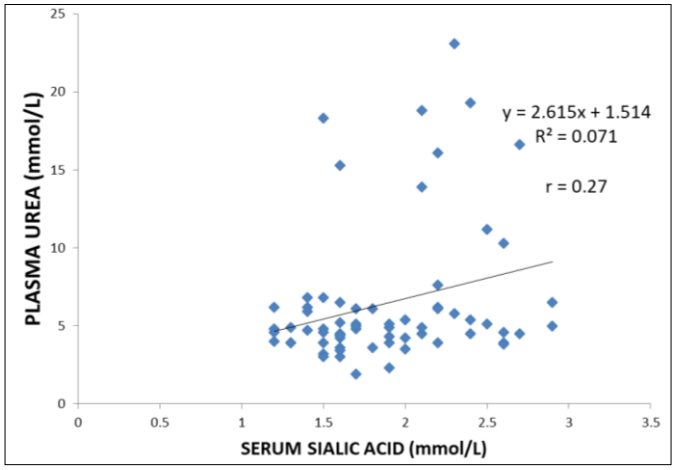

Fig 5: Relationship between serum sialic acid and plasma urea in sickle cell anaemia patients (n=68)

Discussion

Patients with sickle cell anaemia can develop special and sometimes life-threatening complications as well as extensive organ damage, reducing both their quality of life and life expectancy [14]. It is therefore necessary that these patients are closely monitored to avoid such complications. Sialic acid is a common terminal sugar unit of the oligosaccharide of glycoproteins and glycolipids which are cell surface constituents. These sialic acids enter the circulation by either shedding or lysis and are of considerable interest because of their potential diagnostic value [15]. This study showed that serum sialic acid in the SCA respondents (1.88 ± 0.96 mmol/L) were significantly lower than in the control subjects (1.93 ± 0.67mmol/L). Ekeke and Ibeh in their study showed a similar decrease in serum sialic acid in sicklers possibly implying that the loss of sialic acid in erythrocyte membrane is reflected in a removal of sialic acid from the circulation [9]. According to Yokoyama et. al., raised serum sialic acid

concentration preceeds onset of microalbuminuria in SCA patients. Therefore, serum sialic acid a marker of acute phase response, may be an early signal of increased risk of vasculopathy [16].

All the SCA respondents had normal plasma urea (6.43 ± 0.54 mmol/L) and creatinine (86.70 ± 6.03 µmol/L). This finding is similar though higher than that obtained (urea, 2.9 ± 0.9mmol/L and creatinine 76.9 ± 26.5 mmol/L) by Bolarinwa *et al* [17]. The higher mean plasma urea and creatinine in males than female SCA subjects is also similar to previous reports of Abdu et. al., who also found higher plasma urea and creatinine in males [18]. The finding of a significantly higher mean serum sialic acid in female (1.93 ± 0.09 mmol/L) than male (1.80 ± 0.09) SCA subjects corroborates findings of Crook et. Al [19]. The reason for this sex difference is not clear. However it is speculated by Donahue and Orchard (1992) that a higher acute phase response in women with diabetes may reflect the fact that diabetic women loose the protection from cardiovascular diseases [20].

Amongst the SCA respondents in this study, a non- significant positive correlation was found between SSA and blood pressure, BMI and WHR and these could be strong predicting factors for cardiovascular morbidity in SCA. Crook et. al., in their study found an association between serum sialic acid and wait to hip ratio, blood pressure, hyperlipidaemia, glycaemic control, and smoking which are known risk factors for the development of diabetic micro vascular and macro vascular disease [19]. Regression and correlation analysis showed a significant positive correlation between serum sialic acid and plasma urea and creatinine in SCA respondents. This shows that serum sialic acid is significantly associated with renal dysfunction as well as other cardiovascular complications in SCA patients.

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