Journal of Advances in Medicine and Medical Research

26(11): 1-9, 2018; Article no.JAMMR.42188
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Serum and Urine Sialic Acid in Sickle Cell Nephropathy

O. E. Onovughakpo-Sakpa[1], E. S. Idogun[1], E. Ayinbuomwan[1*] and E. C. Onyeneke[2]

[1]Department of Chemical Pathology, College of Medicine, Unversity of Benin, Benin City, Nigeria.
[2]Department of Biochemistry, University of Benin, Benin City, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OEOS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ESI and EA managed the analyses of the study. Author ECO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/42188
Editor(s):
(1) Dr. Dean Markic, Assistant Professor, Department of Urology, University Hospital Rijeka, Croatia.
Reviewers:
(1) Vlachaki Efthymia, Aristotle University of Thessaloniki, Greece.
(2) Priscila Bacarin Hermann, Universidade Federal do Paraná, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/25227

Original Research Article

Received 25th March 2018
Accepted 8th June 2018
Published 22nd June 2018

ABSTRACT

Background: Renal manifestations of sickle cell anaemia range from functional abnormalities to gross anatomic alterations of the kidneys. As people with sickle cell anaemia (SCA) grow older, the kidneys may progress to end-stage renal disease if proper monitoring of renal function is not done.
Aim: The aim of this study is to determine the serum and urine sialic acid levels in SCA patients and correlate with plasma urea and creatinine.
Methods: Venous blood and fresh urine samples were collected from participants and the standard Ehrlich method was used in the analysis of serum and urine sialic acid. The modified Jaffe method was used in the analysis of plasma and urine creatinine and the Urease Berthelot method for plasma urea.
Results: A total of 98 respondents participated in the study consisting of 68 SCA patients and 30 control subjects. The mean age was 28.35 ± 0.42 years for SCA subjects and 33.12 ± 1.14 years for control subjects and most of the SCA subjects were females (M:F = 1 : 1.6). The serum sialic

**Corresponding author: E-mail: ekiyeayinbuomwan@gmail.com;*

acid (SSA) level was 1.88 ± 0.96 mmol/L for SCA subjects and 1.93 ± 0.67 mmol/L for controls and it was found to be non-significantly (P =0.81) higher in SCA subjects with microalbuminuria (1.89 ± 0.07 mmol/L) than in those with normoalbuminuria (1.86 ± 0.10 mmol/L). However, the mean urine sialic acid creatinine ratio (USACR) was higher in SCA subjects (169.39 ± 13.59 mmol/mol) than in the control subjects (60.52 ± 3.39 mmol/mol) and this was statistically significant (P< 0.05). Also, the mean USACR was significantly (P < 0.05) higher in SCA subjects with microalbuminuria than in those with normoalbuminuria.

Conclusion: Serum sialic acid (SSA) is low while USACR is high in SCA patients. Hence, monitoring of serum and urine sialic acid in patients with sickle cell anaemia will be important in detecting early onset of sickle cell nephropathy.

Keywords: Serum Sialic Acid (SSA); Urine Sialic acid Creatinie Ratio (USACR); Sickle Cell Anaemia (SCA); Albumin Creatinine Ratio (ACR).

1. INTRODUCTION

Sickle cell anaemia (SCA) is a genetic life-long blood disorder characterized by red blood cells that assume an abnormal rigid, sickle shape. SCA is particularly common among people whose ancestors come from sub-Saharan Africa, India, Saudi Arabia and Mediterranean countries [1]. Frequency of the carrier state determine the prevalence of SCA at birth. For example, in Nigeria, 24% of the population are carriers of the mutant gene and prevalence of SCA is about 2%. Hence in Nigeria alone, about 150,000 children are born annually with sickle cell anaemia [1]. Globally, sickle cell trait affects approximately 5.5 million neonates and 312 thousand neonates were born with sickle cell anaemia in 2010 [2]. In the coming decades, this worldwide burden is expected to markedly increase [3]. Sickling decreases the cells' flexibility and results in a risk of various complications one of such is renal insufficiency [4]. As more patients with SCA reach the third and fourth decades of life, the incidence of clinically apparent renal insufficiency will increase [5]. Renal manifestations of SCA can range from haematuria and abnormal tubular function to massive proteinuria and end stage renal disease [6]. Proteinuria particularly albuminuria, is the hallmark of glomerular injury which progress to sickle cell nephropathy. Microalbuminuria which is used to define chronic kidney disease is known as albumin excretion rate ≥ 30 mg/24 hrs or albumin-creatinine ratio (ACR) ≥30 mg/g (≥3.0 mg/mmol) [7]. Early detection therefore, of microalbuminuria by screening allows for intervention aimed at preventing or slowing the progress of sickle cell nephropathy.

Sialic acid, a generic term for a family of acetylated derivatives of neuraminic acid, is an essential component of glycoproteins and glycolipids [8]. Sialic acid-rich glycoprotein is found mainly in cell membranes and elevated levels may indicate excessive cell membrane damage, but more specifically for cells of vascular tissue. Damage to vascular tissue leads to ischaemia which most affects the small blood vessels, particularly in the retina, kidneys, heart and brain [9]. In addition, sialic acid contributes to the maintenance of the negative charge of the renal glomerular basement membrane [10]. Sialic acids are found in the glomeruli, and the tubules and their leakage into the urine has been suggested to be a marker or an indicator of injury to the nephrons and renal papilla [11].

Research has shown that the concentration of sialic acid in serum is elevated in pathological states when there is tissue damage, tissue proliferation and inflammation. Studies have also indicated that vascular permeability is regulated by sialic acid moieties [12]. The aim of this study is to determine serum sialic acid levels in sickle cell anaemia patients and correlate it with plasma urea and creatinine as well as microalbuminuria. This study therefore intends to screen for serum sialic acid (SSA) and urine sialic acid (USA) in sickle cell anaemia patients with and without microalbuminuria.

2. PATIENTS 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. SCA patients were grouped into those with microalbuminuria and those without microalbuminuria based on ACR ≥ 3.0 mg/mol.

2.1 Sample Collection

In the morning (at about 9:00am) of their appointment at the Sickle Cell Centre, fresh urine specimen were collected from participants into sterile bottles and used for immediate determination of urinary albumin/creatinine ratio and urinary sialic acid. In addition, 7.0 ml of venous blood was obtained from the cubital fossa using 10.0 ml syringe. About 4.0 mls of blood was dispensed into lithium heparin bottle for plasma urea and creatinine estimation while 3.0 mls of the specimen was centrifuged at 3000 g after allowing it to clot. Plasma / serum was then harvested and stored at 2 - 8®C for about 48 hrs before being analyzed for plasma urea and creatinine and serum sialic acid.

2.2 Biochemical Analysis

Plasma urea was estimated by urease – Berthelot method as outlined by Weatherburn [13], urine and plasma creatinine by the modified Jaffe's method as outlined by Spierto et al. [14], urine albumin was analysed by the Lowry method and serum and urine sialic acid were analysed using the Standard Ehrlich method [15].

2.3 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.'

3. RESULTS

A total of 98 respondents participated in the study. The number of SCA patients were 68 (26 males and 42 females) while the controls (non-sickle cell anaemia) were 30 consisting of 15 males and 15 females. Fig. 1 shows the frequency of age distribution of patients with and without microalbuminuria. Of the SCA patients with microalbuminuria 31(59.6%) and 10 of the patients without microalbuminuria (62.5%) were between the ages 18 – 27 years. Both groups have 2 patients in the age class of 38 – 47 years.

Fig. 2 shows serum sialic acid concentration in SCA patients and in controls. The SCA patients had lower levels of serum sialic acid (SSA) (1.89 ± 0.07 mmol/L) in those with microalbuminuria and 1.86 ± 0.10 mmol/L in those without microalbuminuria as compared to the control group (1.93 ± 0.67 mmol/L).

The serum sialic acid (SSA) level from the study was found to be 1.93 ± 0.67 mmol/L for control subjects and 1.88 ± 0.96 mmol/L for the SCA patients (Table 1). The results also showed that the ACR was 2.19 ± 0.10 mg/mmol and 4.50 ± 0.24 mg/mmol for control subjects and SCA patients respectively, while plasma urea was 3.33 ± 0.16 mmol/L for control subjects and 6.43 ± 0.54 mmol/L for SCA patients. The plasma creatinine was found to be 73.98 ± 2.95 µmol/L for subjects and 86.70 ± 6.03 µmol/L for SCA patients. The mean SSA was found to be significantly (P=.04) lower in the SCA respondents when compared to the controls while the ACR and plasma urea were significantly (P<.05) elevated in the SCA patients than in the controls. Also, urinary sialic acid creatinine ratio (USACR) was found to be significantly higher (P< .05) in SCA (169.39 ± 13.59 mmol/mol) than in controls (60.52 ± 3.39 mmol/mol) (Table 1).

Table 2 shows mean SSA, ACR and USACR in the different age classes in SCA subjects. The results showed a non- significant statistical difference in SSA (P= 0.28), and ACR (P=0.56); with mean values in the second decade (1.81 ± 0.07 mmol/L and 4.42 ± 0.28 mg/mmol) being lower than in the third decade (2.05 ± 0.11 mmol/L and 4.83 ± 0.58 mg/mmol) and a decrease in the fifth decade (1.83 ± 0.21 mmol/L and 3.40 ± 0.34 mg/mmol). However, there was a numerical increase in mean USACR through the second (163.96 ± 17.28 mmol/L) to the fifth (193.38 ± 18.95 mmol/L) decade.

Table 3 shows some biochemical characteristics of SCA patients with and without microalbuminuria. The plasma ACR and USACR were significantly higher (P<.05) in those with microalbuminuria (5.34 ± 0.26 mg/mmol and 197.06 ± 18.09 mmol/mol) than in those without microalbuminuria (2.63 ± 0.10 mg/mmol and 108.77 ± 7.88 mmol/mol). However there were

no significant differences (*P*> .05) in the plasma urea and creatinine and SSA between those with microalbuminuria (6.70 ± 0.69 mmol/L, 85.15 ± 6.19 µmol and 1.89 ±0.07 mmol/L respectively) and those without microalbuminuria (5.82 ± 0.69 mmol/L, 90.16 ± 13.99 mmol/L and 1.86 ± 0.10 mmol/L).

Table 4 showed that in SCA subjects with microalbuminuria, there was a significant correlation between USACR and SSA (*r* =.43, *P* =.001) while there was a non - significant correlation between USACR and plasma urea (*r* =.25, *P*=.10) and creatinine (*r*=.14, *P* =.34), and ACR (*r*=.27, *P*=.08).

It was observed that there was a significant (*P*<.05) increase in mean fractional excretion of sialic acid in SCA subjects (0.78 ± 0.08%) than in the control (0.29 ± 0.04%). However there was a non significant (*P*=.07) increase in mean fractional excretion of sialic acid in SCA without microalbuminuria (1.04 ± 0.18%) and in those with microalbuminuria (0.67 ± 0.07%) [Table 5].

Figs. 3, 4, 5 and 6 show the relationship between USACR and plasma urea and creatinine, ACR and SSA in SCA patients. There was a positive correlation between USACR and plasma urea (*r*=.27. *P*=.03; Fig. 3), and creatinine (*r*= 0.13, *P*<.05; Fig. 4) as well as ACR (*r* =.43, *P* <.05; Fig. 5) and SSA (*r* =.37, *P*< .05; Fig. 6).

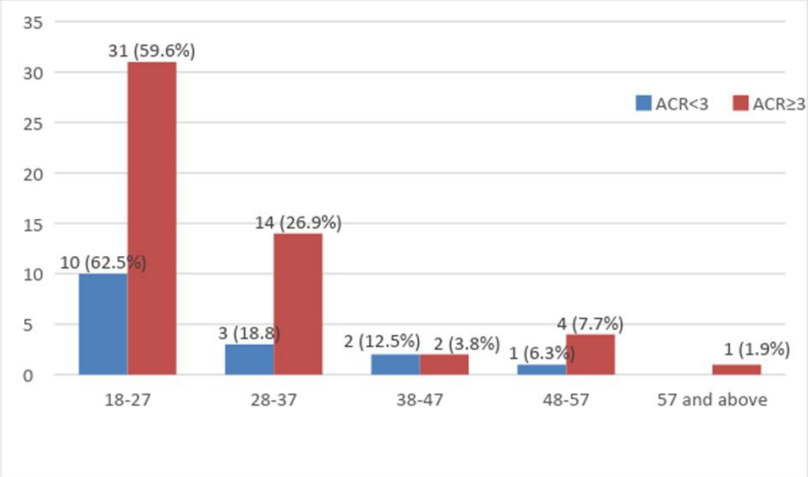

Fig. 1. Age group distribution of SCA patients based on ACR

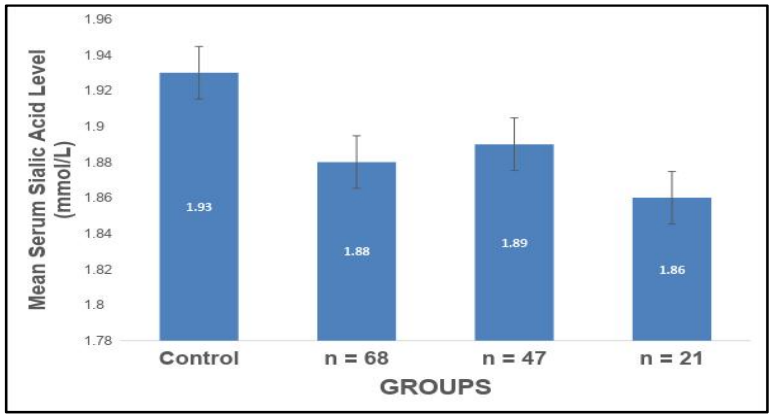

Fig. 2. Mean serum sialic acid concentration in the different study groups

A – Control (non sickle cell anaemia subjects; n = 30)
B – Sickle cell anaemia subjects (n = 68)
C – Sickle cell anaemia subjects with microalbuminuria (n = 52)
D – Sickle cell anaemia subjects without microalbuminuria (n = 16)

Table 1. Plasma urea and creatinine, ACR, SSA and USACR in subjects (non SCA) and SCA patients

	Subjects (non-SCA anaemia) patients(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 ± 6.03	0.06
SSA (mmol/L)	1.93 ± 0.67	1.88 ± 0.96	0.04
ACR (mg/mmol)	2.19 ± 0.10	4.50 ± 0.24	<0.001
USACR (mmol/mol)	60.52 ± 3.39	169.39 ± 13.59	<0.001

Table 2. Mean SSA, USA, USACR, Urinary albumin and ACR in the different age classes in SCA patients

	18 – 27 N = 42	28-27 N= 17	38 – 47 N= 4	48 – 57 N= 5	P value
SSA (mmol/L)	1.81 ± 0.07	2.05 ± 0.11	2.00± 0.09	1.83 ± 0.21	0.283
USACR (mmol/mol)	163.96±17.29	175.76±22.90	185.00±23.18	193.38±18.95	0.942
ACR mg/mmol	4.42 ± 0.28	4.83 ± 0.58	4.48 ± 1.11	3.40 ± 0.34	0.555

Table 3. Biochemical characteristics of SCA patients with and without microalbuminuria

	SCA patients with microalbuminuria (ACR≥3.5 mg/mmol, n = 47)	SCA patients without microalbuminuria (ACR < 3.5 mg/mmol, n = 21)	p-value
Plasma urea (mmol/L)	6.70 ± 0.69	5.82 ± 0.69	0.43
Plasma creatinine (µmol/L)	85.15 ± 6.19	90.16 ± 13.99	0.75
ACR (mg/mmol)	5.34 ± 0.26	2.63 ± 0.10	0.001
SSA (mmol/L)	1.89 ± 0.07	1.86 ± 0.10	0.81
USACR (mmol/mol)	197.06 ± 18.09	108.77 ± 7.88	<0.001

Table 4. Correlation between USACR and clinical parameters in SCA patients with ACR ≥ 3.5 (n = 47)

Biochemical parameter	R – value	P – value
Urea (mmol/L)	0.249	0.10
Creatinine (µmol/L)	0.14	0.34
ACR (mg/mmol)	0.265	0.08
SSA (mmol/L)	0.433	0.00

Table 5. Mean fractional excretion of sialic acid in the various study groups in SCA patients and subjects patients

Mean fractional excretion of sialic acid in the various study groups (%)		P – value
Study (n = 68) 0.783 ± 0.077	Subjects (n =30) 0.289 ± 0.038	< 0.001
ACR ≥ 3.0 (n= 52) 0.670 ± 0.073	ACR < 3.0 (n= 16)1.037 ± 0.180	0.070

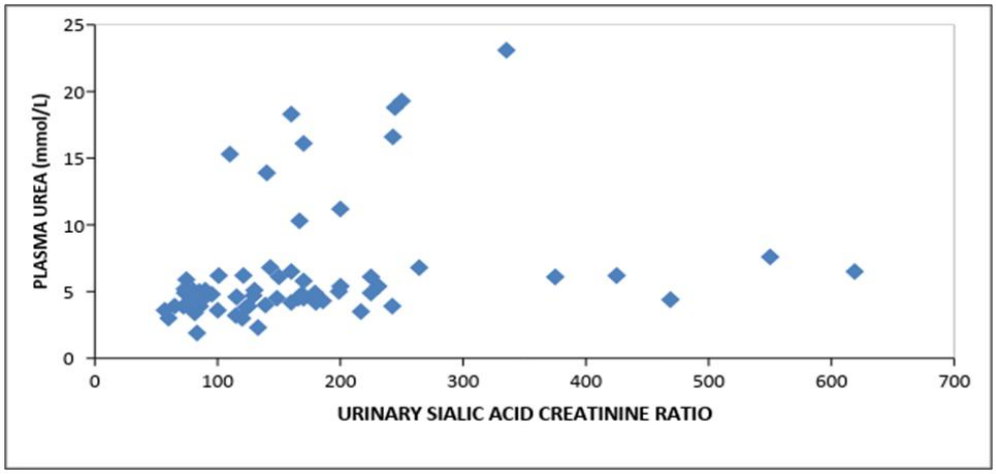

Fig. 3. Relationship between urinary sialic acid creatinine ratio and plasma urea in sickle cell anaemia subjects (n=68)

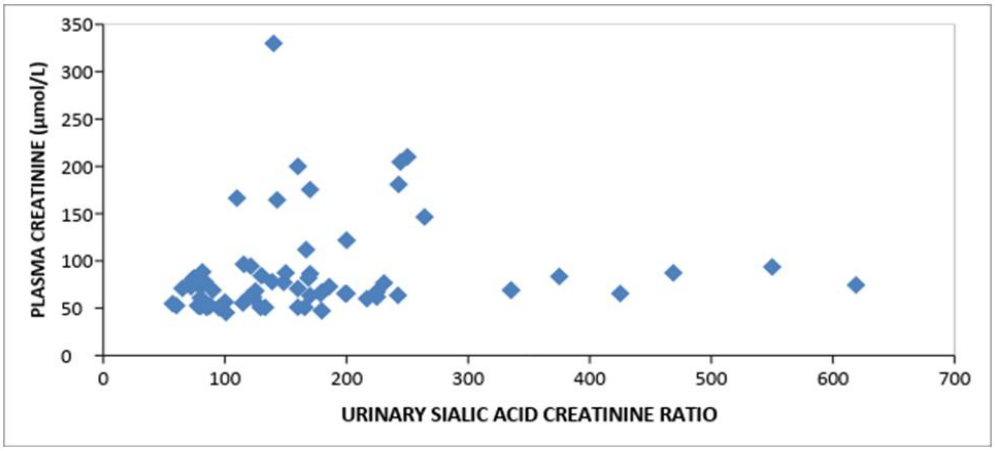

Fig. 4. Relationship between urinary sialic acid creatinine ratio and plasma creatinine in sickle cell anaemia sbjects (n=68)

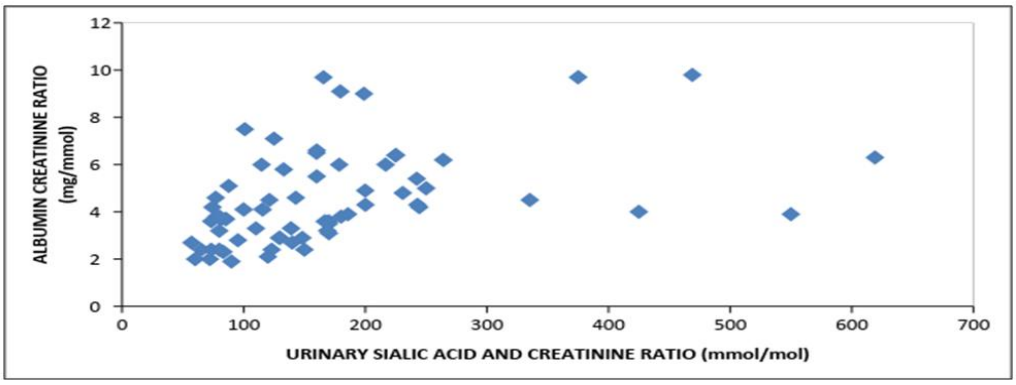

Fig. 5. Relationship between urinary sialic acid creatinine ratio and albumin creatinine ratio in sickle cell anaemia subjects (n=68)

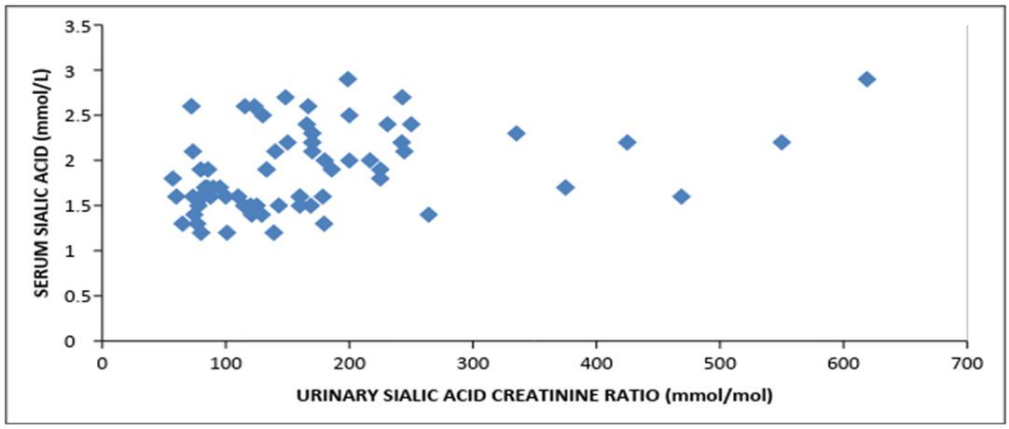

Fig. 6. Relationship between urinary serum sialic acid creatinine ratio and sialic acid in sickle cell anaemia subjects (n=68)

4. DISCUSSION

This study showed that serum sialic acid in SCA patients was lower than in the controls. This is supported by Ekeke and Ibeh who studied sialic acid in sickle cell disease in Port Harcourt, Nigeria and concluded that the loss of sialic acid in erythrocyte membrane is reflected in a removal of sialic acid from the circulation [16]. This study also showed a non-significant increase in serum sialic acid in patients with microalbuminuria than in those without microalbuminuria. According to Yokoyama et al., raised serum sialic acid concentration precedes onset of microalbuminuria, therefore sialic acid a marker of acute phase response may be an early signal of increased risk of vasculopathy [17].

The normal plasma urea and creatinine in the SCA patients as observed in previous studies implies that using plasma urea and creatinine as markers of renal impairment is unrealistic in SCA patients [18,19]. The finding that urinary sialic acid excretion was higher in SCA patients with microalbuminuria than those without microalbuminuria could be due to the fact that sialic acid is filtered by renal glomeruli but not absorbed by human kidney epithelial cells as reported by Seppala et al. [20].

This study found that urinary sialic acid excretion increased with age in SCA patients. However previous studies by Fang-Kircher in non-sickle cell anaemia patients, reported a decrease in urinary sialic acid excetion with increasing age in spot urine as well as 24 hr urine in adults and children [21]. This increase in urinary sialic acid with age found in this study may be associated with age related nephropathy present in SCA. According to Ataga et al. [22] proteinuria is age-dependent in sickle cell disease. When assessed either as microalbuminuria or macroalbuminuria, proteinuria occurs in up to 27% of patients in the first three decades and up to 68% of older patients.

The finding that high plasma urea and creatinine levels were associated with increased urinary sialic acid excretion show that the presence of increased urinary sialic acid excretion is associated with progression of renal dysfunction in SCA patients. This also suggests that increased urinary sialic acid excretion could be an index of the degree of glomerular damage and could be used as a diagnostic tool. It serves as a sensitive early indicator of the adverse effects of SCA on the kidney and is a reliable predictor of renal course.

This study showed a significant increase in mean fractional excretion of sialic acid in SCA patients. According to Seppala et al. [20] parts of excreted sialic acid are drawn from the renal cells themselves or from cells of the urinary tract and various types of renal cells have been reported to synthesize free and bound sialic acid. There was also an increase in mean fractional excretion of sialic acid in non-albuminuric SCA patients than in those with microalbuminuria [20]. This could be due to the presence of progressive renal insufficiency in patients with microalbuminuria. The study also revealed that SSA and urinary sialic acid excretion are associated with microalbuminuria. Microalbuminuria and increased sialic acid excretion indicates the presence and progression of renal insufficiency.

5. CONCLUSION

In conclusion, regular monitoring of SSA, urinary sialic acid and ACR is advocated in SCA patients to delay progression of renal dysfunction to end stage renal disease.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization (WHO). Sickle cell anaemia. 59th World Health Assembly Provisional Agenda. Item A 59/9; 2006.
2. Peil FB, et al. Global epidemiology of sickle haemoglobin in neonates: A contemporary geostatistical model-based map and population estimates. Lancet. 2013;381:142-151.
3. Peil FB, Hay SY, Gupta S, Weatherall DJ, William TN. Global burden of sickle cell anaemia in children under five, 2010 – 2050: Modeling based on demographics, excess mortality and interventions. PLoS Med. 2013;10:e1001484.
4. Platt OS, Brambilla DJ, Rosse WJ, Milner PF, Castro O, Steinberg MH, Klug PP. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med. 1994;330(23):1639–1644.
5. Wong WY, Elliot-Mills D, Powars D. Renal failure in sickle cell anaemia. Hematol Oncol Clin North Amer. 1996;10:1321–1333.
6. Powars DR, Chan LS, Hiti A, Ramicone E, Johnson C. Outcome of sickle cell anaemia: A 4-decade observational study of 1056. Medicine (Baltimore). 2005;84(6): 363-376.
7. KDIGO 2012. Clinical practice guideline for the evaluation and management of chronic kidney disease, summary of recommendation statements. Kidney Int. Suppl. 2013;3:5–14.
8. Ajit V, Schauer R. Essentials of glycobiology. Cold Spring Harbor Laboratory Press; 2009.
9. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care. 2004;27(3):813–823.
10. Blau EB, Haas JE. Glomerular sialic acid and protein in human renal disease. Lab Invest. 1973;28:477–481.
11. Goyer RA, Weinberg CR, Vietery WM, Miller CR. Lead induced nephrotoxicity: Kidney calcium as an indicator of tubular injury. In: Bach P, Lock EA, (eds). Nephrotoxicity: *In vitro* to *In vivo*, animals to man. New York: Plenum Press. 1989;11–20.
12. Crook MA, Pickup JC, Lumb PJ, Georgino F, Webb DJ, Fuller HJ. Relationship between plasma sialic acid concentration and microvascular and macrovascular complications in type 1 diabetes. Diabetes Care. 2001;24:316–322.
13. Weatherburn MW. Colorimetric urease – Berthelot method of urea estimation. Annal. Chem. 1967;39:971.
14. Spierto FW, McNeil ML, Burtis CA. The effect of temperature and wavelength on the estimation of creatinine with the Jaffe procedure. Clin Biochem. 1979;12:18–21.
15. Werner L, Odin L. On the presence of sialic acid in certain glycoproteins and gangliosides. Acta Soc Med Ups. 1952;57: 230–241.
16. Ekeke GI, Ibeh GO. Sialic acid in sickle cell disease. Clin Chem. 1988;34(7):1443–1446.
17. Yokoyama H, Jensen JS, Myrup B, Mathiensen ER, Ronn B, Deckter JA. Raised serum sialic acid concentration precedes onset of microalbuminuria in IDDM. Diabetes Care. 1996;19:435–440.
18. Bolarinwa RA, Akinlade KS, Kuti MA, Olawale OO, Akinola NO. Renal disease in adult Nigerians with sickle cell anaemia: A report of prevalence, clinical features and risk factors. Saudi J Kidney Dis Transpl. 2012;23(1):171–175.
19. Abdu A, Emokpae MA, Uadia PO, Kuliya-Gwarzo A. Proteinuria among adult sickle

cell anaemia patients in Nigeria. Ann of Afr Med. 2011;10(1):34–37.

20. Seppala R, Renuld M, Bernardini I, Tietze F, Gahl WA. Renal handling of free sialic acid in normal humans and patients with Salla disease or Renal disease. Lab Invest. 1990;63(2):197–203.

21. Fang-Kircher SG. Comparison of sialic acids excretion in spot urines and 24-hour-urines of children and adults. Eur J Chem Clin Biochem. 1997;35(1):47–52.

22. Ataga KI, Derebail VK, Archer DR. The glomerulopathy of sickle cell disease. Am. J. Hematol. 2014;89:907-914.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25227