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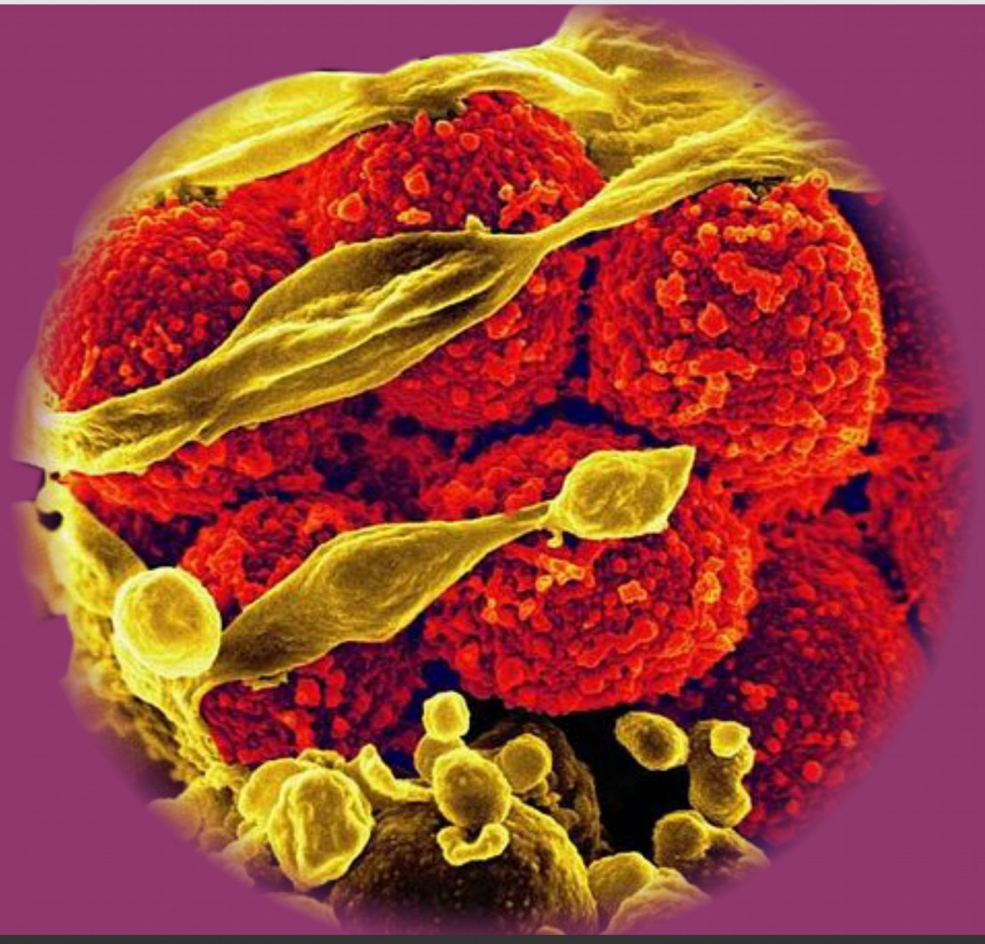
NIGERIAN JOURNAL OF HAEMATOLOGY

Journal of the Nigerian Society for Haematology & Blood Transfusion



ISSN: 2635-3024 **VOL. 2 NO. 1, MARCH 2019**

Special Feature:
Guidelines for the Management of Venous Thromboembolism in Nigeria.

ORIGINAL RESEARCH ARTICLE

Evaluation of Renal Arterial Resistivity Index and Some Biochemical Parameters in Sickle Cell Disease: A Preliminary Study for Early Detection of Renal Impairment

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ABSTRACT

Background: The Doppler Resistive Index (RI) measures intrarenal arterial resistance. It is increased in a number of kidney diseases and considered a marker of renal function, which could serve as an early radiologic predictor of renovascular changes in sickle cell disease (SCD).

Aims and Objective:

This study evaluated selected biochemical markers of renal functionand renal Doppler RI in a cohort of Nigerian adults with SCD and Hb AA controls.

Materials and Methods:

Forty-four Hb SS patients in steady state (M:F = 1.2:1; median age is 24.5) and 22 Hb AA age and sex-matched controls were recruited consecutively into the study. All had serum biochemical tests including serum cystatin-C, serum kidney injury molecule-1 and serum creatinine while urinary albumin-creatinine ratio was also evaluated. The right renal artery Doppler RI was also evaluated in the segmental/interlobar arteries while relevant non-parametric tests were used to compare the biochemical and Doppler parameters between the subjects and the controls.

Results:

Serum creatinine ranged from subnormal to mildly elevated levels in patients with Hb SS (33-245µmol/l) and the median (range) renal arterial Doppler RI was 0.70 (0.49-0.81). The other parameters evaluated were significantly higher in patients relative to the controls. Of the biochemical parameters, urinary albumin- creatinine ratio showed a weak but statistically significant positive correlation with renal arterial Doppler RI (r=0.329; *p*= 0.029), while serum cystatin-C and kidney injury molecule-1 had no correlation (r = 0.152 and 0.188 respectively; *p* = 0.324 and 0.22).

Conclusion:

Renal arterial Doppler Resistivity Index has a linear relationship with urinary albumin-creatinine ratio and could therefore be a potential marker of early renal impairment in patients with SCD.

Keywords: Sickle cell disease, renal arterial Doppler resistive index, cystatin-C, serum kidney injury molecule 1, urinary albumin-creatinine ratio.

INTRODUCTION

Sickle cell disease (SCD) is a common hereditary haemoglobinopathy. It is a multisystem disorder affecting almost every organ-system. It often results in renal dysfunction leading to sickle nephropathy in the later stages. Sickle cell nephropathy is indicated by sickled erythrocytes, with the consequent effects of decreased medullary blood flow, ischemia, microinfarct and papillary necrosis. [1] Another possible cause of nephropathy in SCD patients is the chronic abuse of analgesics from treatment of chronic pain due to vaso-occlusion. [2,3] There is, however, paucity of data on the abuse of analgesics in SCD patients.

In a cohort of patients with SCD studied in South-West Nigeria, it was noted that 50% had albuminuria while 31% had glomerular hyperfilteration. Among the latter, 25%, 42% and 3% had stages1, 2 and 3 chronic kidney diseases respectively. [4] A related study assessing 374 adult and children SCD patients observed a significant renal impairment in 37% of the patients in the cohort. [5] The use of traditional and some novel biochemical markers in SCD patients including serum creatinine, cystatin-C (cys-C), urinary albumin-creatinine ratio (ACR), urinary kidney injury molecule 1 (KIM-1), N - a c e t y l - b - D - glucosaminidase (NAG) among others to prognosticate nephropathy has been described in the literature. [6-9] Also, researchers have evaluated the utility of renal arterial Doppler ultrasound parameters especially RI and pulsatility index (PI). [7] Doppler ultrasonography is a non-invasive and relatively cheap method for assessing RI which invariably measures the renal haemodynamic alterations and reno-vascular changes in patients with SCD.[7, 10] Aikimbaev *et al* [10] reported an increased renal vascular resistance among the patients with SCD compared to age-matched controls using renal arterial Doppler indices.

Renal arterial Doppler RI and biochemical indices were measured in patients with homozygous SCD and a positive relationship between renal RI and biochemical markers (namely cys-C, KIM-1, creatinine, urine ACR) used in the evaluation of renal impairment among patients with SCD in steady state was hypothesized.

MATERIALS AND METHODS

Patients Sampling and Study Design

The prevalence of homozygous sickle cell disease (Hb SS) is 1-3% for all ethnic groups in Nigeria. [11] The sample size was determined by using a prevalence of 3% at a standard normal deviate of 1.96 (95% confidence interval) and the degree of accuracy was set at a p-value of < 0.05. [12]

This cross-sectional observational study included 44 consecutively recruited patients in the steady state aged 16 years and above from April 2014 to March 2015. All the patients were recruited from the Haematology Outpatient Clinic, Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife. Twenty two age and sex-matched apparently healthy volunteers (Hb AA phenotype; confirmed by haemoglobin electrophoresis) among students and hospital workers were included as controls. The study was approved by the Research and Ethics Committee, OAUTHC, Ile-Ife,(Protocol number: ERC/2013/03/11) and carried out in compliance with the Declaration of Helsinki of 1964 and its subsequent revisions.

Socio-demographic information obtained from all patients and controls included age, sex, weight, height, use of analgesics, number of crises/year and transfusions/year. The body mass index (BMI) was calculated and clinical examination was conducted to exclude patients with congenital urogenital anomalies. All patients with urinary tract infection, diabetes mellitus, human immunodeficiency virus infection and hypertension (systolic blood pressure >125mmHg and diastolic blood pressure >75mmHg) [4], massive oedema, dyslipidemias, on oral contraceptive pills and adrenergic drugs, on dialysis, on medications that may interfere with renal function such as cimetidine, probenecid and ACE inhibitors and smokers were also excluded. None of the patients was on hydroxyurea.

Sample collection and laboratory analysis

Venous blood samples were collected after an overnight fast (12 – 14 hours) into plain specimen bottles, centrifuged at 3000g for 5 minutes to separate the serum, which was collected and stored at -20℃. The serum was allowed to thaw before being analysed for cys-C and KIM-1 using sandwich enzyme linked immunosorbent assay method (ELISA; Aviscerabioscience Company Inc., CA, USA). Biochemical tests were also conducted to rule out diabetes mellitus and dyslipidemia (Point of Care Cardiochek PA analyser; Polymer

Technology Systems, Inc., USA). Micro-albuminuria in early morning urine samples was measured using a point of care Clinitek status test kit (Siemens Healthcare Diagnostics Inc., NY, USA). Presence of microalbuminuria was considered when albumin excretion was in the range of 30-300 mg/dl and/or gross albuminuria when it was more than 300 mg/dl. [13] A urinary strip test was also used to exclude UTI.

Renal Arterial Doppler Ultrasound

Renal arterial Doppler ultrasound was performed on a colour flow Doppler machine (Mindray DC-7, Shenzhen Mindray Bio-medical Electronics Co. Ltd, Shenzhen, China), using 3.5-MHz curvilinear probe. All study participants were examined in the supine position; left lateral decubitus for the right kidney and right lateral decubitus for the left kidney to exclude renal anomalies. Doppler sonography was performed using the non-compression technique on the kidneys. Doppler parameters recorded were RI and PI for all the participants. However, since RI has been shown to be a less variable index than PI, [7] it is the only Doppler sonographic parameter used in the final analysis. All patients and controls were normotensive at the time of renal artery sonography. Doppler parameters were obtained for segmental or interlobar arteries. Three readings from the arteries in the upper pole, interpolar, and lower pole regions were taken and the average values of parameters were recorded for the right kidney. Renal arterial Doppler RI>0.7 was considered abnormal. [13] All readings were obtained by the same radiologist, who was blinded to the status of the participants, with over 10 years' experience, to eliminate inter-observer variability and bias.

Statistical analysis

For comparison between groups, *P*-values were calculated using independent samples. Mann-Whitney U test was used for comparison between groups and chi-squared tests for continuous and categorical variables respectively. A *P*-value ≤ 0.05 was considered significant. Statistical Package for the Scientific Solutions (SPSS version 20.0; 2017) was used for data analysis.

RESULTS

The 44 patients with Hb SS were controlled for age and sex using 22 apparently healthy Hb AA controls. The median age for both patients (24.5 years; range: 16-47 years) and controls (24.5 years; range: 17-33 years) was not significantly different (P = 0.620). Table 1 shows the characteristics of the 44 patients (M: F = 1.1: 1) and the 22 controls (M: F = 1.2: 1), while Table 2 shows some clinical and laboratory parameters of the 44 patients. About one-third of the patients (34.1%) reported 1-2 VOCs per year while 0-1 transfusion per year was reported in 79.6%. The median haematocrit was 25% (range: 15 - 32%), while the median WBC was 9,500 cells/mm^3(range: 3,300-20,400 cells/mm^3).

Table 3 compares the biochemical and Doppler ultrasonographic markers of renal function among patients and controls. Serum creatinine ranged from subnormal to mildly elevated levels in patients. Of the 44 patients, 13 (29.5%) had creatinine values above the upper limit of normal (60-106umol/l), the median value was 152 umol/l (range: 109-245 umol/l).

Serum cys-C and KIM-1 were also significantly elevated in patients when compared to the controls (*P* < 0.001). Similarly, urinary ACR (P = 0.006) and renal arterial Doppler RI (P< 0.001) were significantly elevated in patients (0.70 [0.49-0.81]) when compared to controls (0.61 [0.5-0.68]) respectively.

Non-parametric correlation of renal arterial Doppler RI and some renal biochemical markers inpatients are presented in the Table 4. Only urinary albumin-creatinine ratio showed a weak, but statistically significant positive correlation with renal arterial Doppler RI (r = 0.329; *P* = 0.029).

Table 1: Characteristics of 44 patients with Hb SS and 22 Hb AA controls

Variable	Hb SS n=44	Hb AA n=22	P-value
Age (years)			
Median (range)	24.5 (16-47)	24.5 (17-33)	0.620
Age group	**n (%)**	**n (%)**	
<20 years	11 (25.0)	4 (18.2)	-
20 - 29 years	16 (36.4)	15 (68.2)	
30 - 39 years	14 (31.8)	3 (13.6)	
≥40 years	3 (6.8)	0 (0.0)	
Gender,	**n (%)**	**n (%)**	
Male	23 (52.3)	12 (54.5)	1.000
Female	21 (47.7)	10 (45.5)	
Height (m)			
Median (range)	1.62 (1.13-1.81)	1.63 (1.46-1.93)	0.822
Weight (Kg)			
Median (range)	49.0 (25-72)	61.0 (45-91)	<0.001
BMI (Kg/m^2)			
Median (range)	18.1 (14.5-31.2)	22.6 (15.4-30.4)	<0.001
BMI group	**n (%)**	**n (%)**	
Underweight	24 (54.5)	3 (13.6)	**-**
Normal BMI	18 (40.9)	11 (50.0)	
Overweight	1 (2.3)	7 (31.8)	
Obese	1 (2.3)	1 (4.5)	

Independent samples Mann-Whitney U tests
P-value ≤ 0.05 is statistically significant

Table 2: Clinical and laboratory characteristics of 44 HbSS patients

Variable	HbSS n=44
VOC per year	**n (%)**
0-1	9 (20.5)
1-2	15 (34.1)
2-3	11 (25.0)
>3	9 (20.5)
Transfusion per year	**n (%)**
0-1	35 (79.6)
1-2	6 (13.6)
2-3	3 (6.8)
Haematocrit[%;Median (range)]	25.0 (15-32)
WBC x 10^{3}cells/mm^3(Median (range)	9.5 (3.3-20.4)

Key: VOC-vaso-occlusive crisis; WBC-white blood cell count

Table 3. Comparing renal biochemical and Doppler ultrasonographic parameters among patients with HbSS and HbAA controls

Variables	Hb SS n=44 Median (range)	Hb AA n=22 Median (range)	P-value
Serum C reatinine (µmol/l)	87.5 (33-245)	84. 6 (63-104)	0.035
Serum Cystatin-C (mg/l)	4.80 (0.80-21.30)	0.9 (0.4-2.4)	<0.001
Serum KIM-1 (ng/ml)	0.45 (0.17-4.40)	0.12 (0.09-0.25)	<0.001
Urinary ACR (mg/g)	20.0 (3.3-800.0)	12.5 (3.3-30)	0.006
Renal RI	0.70 (0.49-0.81)	0.61 (0.5-0.68)	<0.001

P-value ≤ 0.05 is statistically significant

Table 4: Non-parametric (Spearmann) correlation of renal Doppler resistive index and some biochemical parameters in the patients (n= 44)

Variables	*r*	P-value
Serum Creatinine (umol/l)	-0.120	0.438
Serum CystatinC (mg/l)	0.152	0.324
Serum KIM-1 (ng/ml)	0.188	0.220
Urinary ACR (mg/g)	**0.329**	**0.029***

*Statistically significant

Figure 1 shows two groups of patient with normal (n = 22) and abnormal (n = 22) renal arterial Doppler RI using a cut-off value of 0.7 [7] with the distribution of the selected biochemical parameters across the two groups compared to Hb AA controls presented as box plots. Serum creatinine values were not significantly different among patients with Hb SS with or without abnormal renal arterial Doppler RI (P = 0.8). However, serum cys-C (P = 0.01) and KIM-1 (P = 0.03) showed statistically significant difference across the three groups. The urinary ACR also showed statistically significant difference between controls and patients with abnormal renal arterial Doppler RI only (P = 0.005).

All patients admitted to frequent daily use of non-steroidal anti-inflammatory drugs (NSAIDS) for at least one month. None of the controls used NSAIDS on a chronic basis.

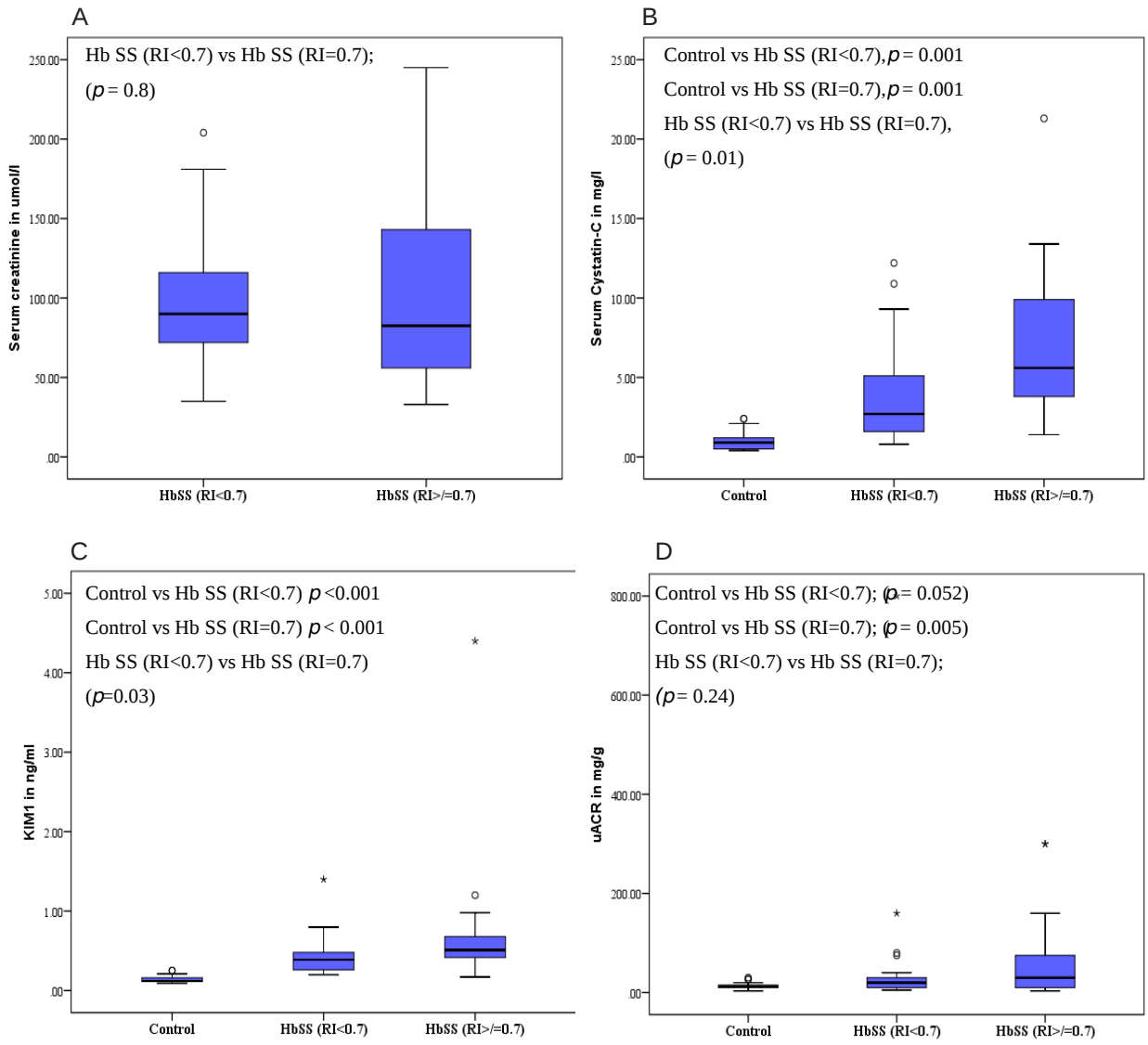

Figure 1. Box plots comparing renal arterial Doppler resistive index (RI) and biochemical parameters among patients with Hb SS and controls (Hb AA). A: represents the plot of serum creatinine (umol/l) against RI; B: represents the plot of serum cystatin-C (mg/l) against RI; C: represents the plot of serum KIM-1 (ng/ml) against RI and D: represents the plot of urinary albumin-creatinine ratio (mg/g) against RI.
P-values are based on Independent sample Mann-Whitney U test.

DISCUSSION

Chronic sickling underlies several mechanisms for kidney injury. The arterial side of the renal microvasculature has low oxygen tension. The hypertonicity and low pH of the renal medulla promote the formation of haemoglobin polymers in the red cells with deformation of the sickled cells, resulting in an increase in the blood viscosity, functional venous engorgement, and interstitial oedema, predisposing the renal microcirculation to ischemia and infarction. [14] In addition to this, patients with SCD have been documented to abuse analgesics due to pain from vaso-occlusion. [2] Nephropathy, which is a slowly progressive disease, can also result from chronic abuse of non-steroidal anti-inflammatory drugs. [3]

A previous study had shown that the use of serum creatinine and its common predictive formulae will result in inappropriate classification of renal function in patients with SCA. [6] Therefore, it was not included among the prime biochemical parameters evaluated in this study. Data obtained in this study showed that serum cys-C and KIM-1; urinary ACR and renal arterial Doppler RI are useful in evaluating early renal impairment in the steady state of SCA. These results also suggest that there is a relationship between the renal arterial Doppler RI and urinary ACR (Table 4; r = 0.329, P = 0.029). Since albuminuria has been documented to be an early marker of sickle cell nephropathy, [15] it could be inferred that a rise in the value of renal arterial Doppler RI may be an early sign of renal impairment in these patients. Lakhkar *et al* also observed that renal arterial Doppler RI and micro-albuminuria were better tools for early detection of renal involvement in children with Hb SS. [16]

Although a non-significant positive correlation was reported in this study between cys-C and KIM-1 with renal arterial Doppler RI values (Table 4), a significant increase in the median values of cys-C and KIM-1 was noted with the lowest values among controls and a linear increase was noted among patients with SCA with normal renal artery Doppler RI values when compared to patients with SCA with high RI values (Figure-1). Asnani *et al* observed that serum cys-C shows strong associations with GFR and albuminuria among patients with SCA and therefore may be a useful screening tool in this population of individuals. [17] This study shows no statistically significant association between serum KIM-1 and albuminuria contrary to the report of Sundaram *et al* who evaluated the utility of some novel biochemical markers for early detection of sickle nephropathy and concluded that urinary KIM-1 and N-acetyl-b-D-glucosaminidase (NAG) had a strong association with albuminuria. [9] This study was limited by the inability to evaluate inulin clearance (the gold standard marker of renal function) [18] or the radioisotopic method as alternative (which is time consuming and very expensive). Notably, measurement of RI by Doppler ultrasonography is available in resource limited settings and it is affordable, but may be subject to intra-operator variability and experience.

CONCLUSION

This study shows a significant relationship between renal arterial Doppler RI and urinary albumin-creatinine ratio. Serum cys-C and KIM-1 values were increased in patients with SCD and normal or abnormal RI values. Renal arterial Doppler RI, in experienced hands could, therefore, be a potential marker for early detection of renal impairment in patients with SCD.

Acknowledgement:

We wish to acknowledge the part-sponsorship of this study by a Grant from the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Osun State, Nigeria.

Disclosure:

There are no conflict of interest to be declared by any of the authors.

Author's Contribution:

All authors contributed to the design of the study and writing of this manuscript. In addition, RAB contributed to the grant application and conducted haematological investigations. OOA conducted the radiological investigations and contributed to grant application . UUO contributed to the grant application. OHO contributed to the grant application. TAA conducted the biochemical investigations. ASA conducted radiological investigations. FAA assisted with the analysis of data.

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ISSN 263530240-5