



Relationship Between Markers of Haemolysis, Disease Severity And Risk For Stroke In Children With Sickle Cell Anaemia In Abeokuta, South-Western Nigeria.

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I. INTRODUCTION

Sickle cell disease (SCD) is an inherited autosomal recessive genetic disorder which arises from the tendency of haemoglobin S (HbS) to polymerize and deform red blood cells (RBCs) into the characteristic sickle shape. (1) The hallmarks of the disorder are chronic haemolytic anaemia and intermittent acute exacerbations of the various sickle cell crises. (1) The clinical manifestations of SCD are, however, multi-systemic and vary tremendously among affected individuals based on the sub-phenotypes.

Neurologic complications are reported to occur in about 25% of affected patients(2) and include: seizures, headaches, visual loss, cognitive impairment, transient ischaemic attacks (TIAs), silent cerebral infarction and strokes (2) Stroke is a devastating complication of SCD, defined by the presence of a focal neurologic deficit lasting for greater than 24 hours and/or abnormal neuroimaging of the brain indicating a cerebral infarct on T2-weighted magnetic resonance imaging (MRI) corresponding to the focal neurologic deficit. (3) Sickle cell anaemia is one of the leading causes of stroke in the paediatric age group,(4) with the highest risk among those with homozygous S disease (HbSS). (5) The clinical consequences of stroke include mortality and long-term impairment of cognitive function and development. (2)

Both ischaemic and haemorrhagic strokes can occur in SCD, though the type varies with age. (3) An ischaemic infarct often occurs as either an overt stroke or a "silent" cerebral infarct. (6) The highest risk of a first overt ischaemic stroke is within the first decade of life; most commonly in children between the ages of two and nine years, with a second peak in adults over 29 years. About 11% of homozygous SCD patients are estimated to suffer a stroke before they are 20 years old. (5,7) Locally, in Nigeria prevalence of stroke in SCD is estimated to be between 6-8% with a case fatality rate of 4.2%. (5)

Ischaemic strokes in SCD are primarily the result of stenosis or occlusion of the anterior intracranial circulation affecting the distal intracranial internal carotid artery (ICA) and/or proximal middle cerebral artery (MCA) and/or the proximal anterior cerebral artery (ACA). (8) Factors which predispose to ischaemic stroke include: haemolysis-induced endothelial injury, low steady-state haemoglobin concentration, high steady-state WBC count, hypertension, prior transient ischaemic attack, silent cerebral infarct present on MRI, recent acute chest syndrome and reticulocytosis. (7) Recent transfusion within 14 days, treatment with corticosteroids and non-steroidal anti-inflammatory drugs are also factors that increase the risk of haemorrhagic stroke in children with SCD (7)

A vital breakthrough in the management of sickle cell-related brain injury is the use of transcranial doppler (TCD) ultrasonography in identifying stroke risk in children with SCD, hence making primary stroke prevention possible. (9-11) TCD measures flow velocities in the intracranial vessels of the circle of Willis. The velocity of blood flow through these vessels is inversely related to vessel diameter; (11) increased blood flow velocity therefore results from focal vascular stenosis, which reduces arterial diameter. Studies (8-10) have shown the efficacy of TCD in identifying stenotic lesions in intracranial vessels, and TCD readings have been demonstrated to be well-correlated with magnetic resonance angiography (MRA) findings. (12) Transcranial doppler ultrasonography thus provides a relatively inexpensive, non-invasive real-time measurement of blood flow characteristics and haemodynamics within the basal arteries of the brain, hence offering the possibility of identifying patients at risk for stroke and prompt institution of preventive measures such as chronic blood transfusion and the use of hydroxyurea, without undue exposures to contrast media and ionizing radiation. (12)



Remarkably, despite the large burden of SCD in the country, there are limited facilities for stroke risk evaluation. A 2013 multi-centre study by Galadanci *et al* (13) indicated that only three centres in the country had facilities for TCD at the time of the report. Although this number has increased significantly over the years (14), there remains a dearth of centres in which TCD is carried out in our practice setting, and patients are often required to travel several miles, often to neighbouring states, to have this crucial investigation done. The financial implications of conducting the investigation are also beyond the reach of many patients in settings like ours, where out-of-pocket healthcare financing is predominant. There is, therefore, a need to identify other readily available clinical and laboratory indices in subjects which may be indicative of risk for stroke in children with SCD in resource-poor settings when evaluation by TCD is not immediately feasible. This study, therefore, set out to evaluate the relationship between markers of haemolysis, disease severity and the risk for stroke as measured by TCD ultrasonography in a cohort of Nigerian children with SCD.

II. MATERIALS AND METHODS

The study was a hospital-based cross-sectional study carried out over a period of nine months at the Departments of Paediatrics of the Federal Medical Centre, Abeokuta (FMCA) and Sacred Heart Hospital (SHH), Lantoro, Abeokuta. Abeokuta is the largest and most populous city in Ogun State, South-West Nigeria.

Study population

The subjects were 75 SCA subjects aged between 2 to 15 years who presented at the haematology clinic during their steady state, and whose parents/guardians consented to their participation in the study. The assents of children older than seven years were also obtained. Children with other SCD variants, as determined by Hb electrophoresis other than HbSS, those on hydroxyurea, and those with established renal and hepatic disorders were excluded.

The age limit was set at 2 and 15 years because children within this age bracket have thinner skulls and large trans-temporal acoustic windows, both of which facilitate skull penetration for TCD ultrasound scans. (11) Although TCD scans have been carried out in infants, their interpretation and clinical utility remain unclear in children aged less than two years. (8) Likewise, the interpretation of TCD findings in children beyond the age of 15 years is also unclear due to inadequate acoustic windows. (11) In addition, the age of 15

years is the upper limit for access to paediatric care at the study centres.

Sampling method

All eligible participants who presented at the haematology clinic for follow-up visits in their steady state during the study period and were hydroxyurea-naïve were consecutively recruited until the estimated sample size was attained. Steady state was taken as that point in time when subjects were not experiencing any acute painful crisis or changes due to therapy and fulfilled the following criteria: No history of an acute painful episode requiring treatment at home or in the hospital, nor an intercurrent illness such as infection, or inflammation for at least four consecutive weeks; and no blood transfusion in the preceding 3 months.

Study instruments.

A proforma detailing socio-demographic, clinical, laboratory and radiological parameters was administered by the researcher. The proforma incorporated the Socio-economic classification model developed by Olusanya *et al* (15), which was used to determine the socioeconomic classes of parents/guardians, as well as the sickle cell disease severity scoring system developed by Adegoke *et al*. (16)

Cellulose Acetate Strips were used for the determination of the haemoglobin phenotypes of subjects by haemoglobin electrophoresis; the Sysmex Xs 1000i Automated Haematology Analyzer Model 16-Parameter Analyzer was used for the haematological profiles. AGAPPE LiquiCHEK LDH-P was used to assay for lactate dehydrogenase, while reticulocyte counts were carried out using the Bio Lab Diagnostic reticulocyte stain kit and viewed with a microscope by an experienced haematologist.

Transcranial Doppler ultrasound scan machine -DWL Doppler box (Scan Med®) with 2 HZ probe was used to evaluate the timed average mean maximum velocities (TAMMV) in the Internal carotid arteries (ICA), middle cerebral arteries (MCA) and anterior cerebral arteries (ACA) of subjects.

Laboratory and Radiologic Procedures

Three millilitres of Ethylene diamine tetra-acetic acid (EDTA) anti-coagulated whole blood was used for haemoglobin electrophoresis, packed cell volume, total white blood cell count, reticulocyte count and lactate dehydrogenase. Transcranial doppler scans were carried out as described in the STOP protocol (11), and the timed



average mean maximum velocity (TAMMV) in the left and right anterior cerebral (ACA), middle cerebral (MCA) and internal carotid (ICA) arteries was determined for each subject. The highest TAMMV value thus obtained in any of these vessels was then used to classify patients according to the stroke prevention in sickle cell disease (STOP) trial protocol as follows: (11)

- . Normal velocities (Standard risk) <170 cm/s
- . Conditional velocity (Intermediate risk) 170–199 cm/s
- . Abnormal velocities (High risk) >200 cm/s

Data collection and analysis

All the information obtained and results from the analysis were recorded in the study proforma. The data obtained was entered into a personal computer and analysed using the Statistical Product and Service Solution version 25. Numeric variables such as age and timed average mean velocities in cerebral vessels were summarized using mean and standard deviations, while variables that failed the normality test were summarized using median and inter-quartile range. Categorical variables such as age groups, gender and socioeconomic class were summarized using frequency tables and percentages.

The association or difference between two categorical variables was analysed using the chi-square (χ^2) test, where the assumption for chi-square was fulfilled and where it was not met, the Fisher exact test was done. The Mann-Whitney U test was used to compare two continuous variables that were not normally distributed, while Spearman correlation analysis was used to test the relationship between two continuous non-parametric variables. The level of significance was set at 5% ($\alpha = 0.05$) while the power of the study was set at 80%.

Ethical considerations

Ethical approval for the study was obtained from the Hospital Research Ethics Committee (HREC) of both hospitals. The purpose and relevance of the study were explained to the parents or caregivers. Written consents and assents were obtained from the parents/caregivers and children aged seven years and above, respectively. Participation was entirely voluntary, and every participant was informed of their right to withdraw at any point they decided to do so. Confidentiality was ensured by allotting serial numbers to each participant, by which they were referenced at all stages of the study. The immediate benefits of participation to the patients included free access to TCD and other routine investigations, which form part of the routine work-up for SCD patients in our practice, while the results of these investigations were communicated to the caregivers in simple terms and made available for decision-making in the management of patients.

III. RESULTS

A total of 75 subjects with sickle cell anaemia (SCA) were enrolled for the study over a nine-month period. Table 1 shows the socio-demographic characteristics of the study participants; the age range of study participants was 2-15 years, with a mean age of 7.35 ± 3.44 years. More than two-thirds (68.0%) were less than 10 years old. The majority (45; 60.0%) of the participants were males, with a male-to-female ratio of 1.5:1. Most of the study participants (64.0%) belonged to the middle socioeconomic class, while 72; 96.0% were of Yoruba ethnicity.

Socio-demographic characteristics of the study participants (Table 1)

	Gender (Subject)		
	Male n (%)	Female n (%)	Total n (%)
Age (years)			
2-5	20(26.7)	07(9.3)	27(36.0)
6-9	15(20.0)	09(12.0)	24(32.0)
10-12	07(9.3)	13(17.3)	20(26.7)
13-15	03(4.0)	01(1.3)	04(5.3)
Total	45(60.0)	30(40.0)	75(100.0)
Socioeconomic class			
Low	09(12.0)	03(4.0)	12(16.0)
Middle	28(37.3)	20(26.7)	48(64.0)
High	08(10.7)	07(9.3)	15(20.0)



Total	45(60.0)	30(40.0)	75(100.0)
Religion			
Christianity	26(34.7)	19(25.3)	45(60.0)
Islam	19(25.3)	11(14.7)	30(40.0)
Total	45(60.0)	30(40.0)	75(100.0)
Tribe			
Yoruba	44(58.7)	28(37.3)	72(96.0)
Igbo	01(1.3)	02(2.7)	03(4.0)
Hausa	00(0.0)	00(0.0)	00(0.0)
Total	45(60.0)	30(40.0)	75(100.0)

The pattern of disease severity among subjects (Figure 1). Using the disease severity score designed by Adegoke *et al*, 84.0% had mild disease, 16.0% had moderate disease, while none of the subjects had severe disease, as demonstrated in Figure 1.

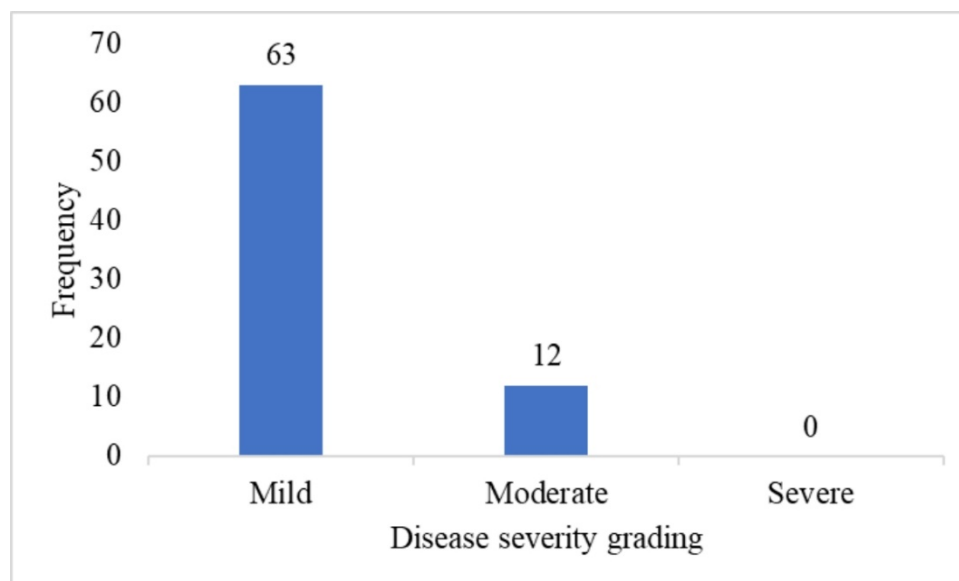


Figure 1: Pattern of disease severity among subjects

The pattern of flow velocity (TAMMV) in the cerebral vessels of subjects(cm/sec)

The range of values of the velocities recorded from the transcranial Doppler scan among subjects shows that the highest velocities were recorded in the left middle cerebral and internal carotid arteries.

Table 2: Pattern of flow velocity (TAMMV) in the cerebral vessels of subjects (cm/sec)

Artery	Right	Left
	Mean± 2SD	Mean± 2SD
MCA	134.7±51.2	141.1±60.2
ACA	103.9±49.4	95.5± 45.4
ICA	136.5±57.6	142.0±60.6

MCA: Middle cerebral artery, ACA: Anterior cerebral artery, ICA: Internal carotid artery

Pattern of transcranial Doppler scan among Subjects; Figure 2



More than two-thirds (53; 70.7%) of the subjects had standard risk TCD with TAMMV <170cm/sec, (16; 21.3%) had conditional risk TCD with TAMMV 170-199cm/sec, while only (6; 8.0%) of them had high-risk TCD with TAMMV \geq 200cm/sec.

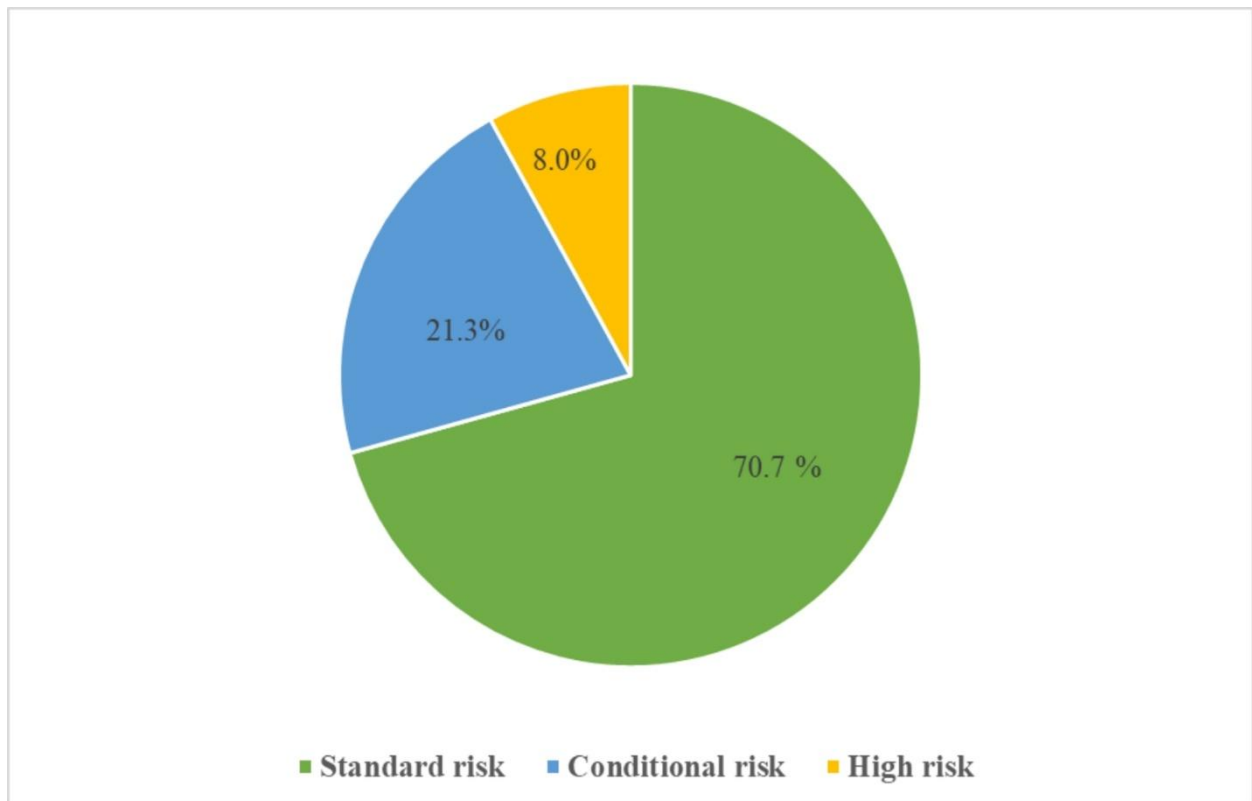


Figure 2: Pattern of transcranial Doppler scan among subjects

Relationship between markers of haemolysis and timed average mean of maximal velocities (TAMMV)

Spearman correlation analysis between markers of haemolysis and the timed average mean of maximal velocities in cerebral vessels among the subjects showed that LDH showed a statistically significant positive correlation with readings from the middle cerebral (right: $r = 0.322$, $P = 0.005$; left: $r = 0.362$, $p = 0.001$) and internal carotid arteries

(right: $r = 0.310$; $p = 0.007$; left: $r = 0.407$; $p = 0.000$). Additionally, packed cell volume also showed a significant negative correlation with readings from the right middle cerebral artery ($r = -0.242$; $p = 0.037$), the right anterior cerebral artery ($r = -0.367$; $p = 0.001$), and the right internal carotid artery ($r = -0.247$; $p = 0.033$). There is no correlation between reticulocyte counts and TAMMV, as shown in Table 3.

Table 3: Relationship between markers of haemolysis and TAMMV

Artery	Markers of haemolysis					
	Packed cell volume (%)		Reticulocyte count (%)		LDH(U/L)	
	R	P	r	P	r	P
MCA(R)	-0.242	0.037*	0.115	0.326	0.322	0.005*
MCA(L)	-0.122	0.297	0.132	0.258	0.362	0.001*
ACA(R)	-0.367	0.001*	0.059	0.613	0.173	0.138
ACA(L)	-0.185	0.111	0.108	0.358	0.032	0.788
ICA(R)	-0.247	0.033*	0.114	0.331	0.310	0.007*
ICA(L)	0.008	0.948	0.243	0.035	0.407	0.000*

* Statistically significant correlation between two variables. R: Right. L; Left

**Relationship between the disease severity and the transcranial Doppler scan conclusion**

The results showed that the population of subjects with conditional and high-risk TCD were more in the moderate disease group than in the mild disease group. However, it should be noted that this pattern was not statistically significant.

Table 4: Degree of disease severity and Transcranial Doppler scan risk classification

Disease severity	Total	Standard risk n (%)	Conditional risk n (%)	High risk (%)	χ^2	p-value
Mild	63.0	45.0(71.4)	13(20.6)	05(7.9)	0.124	0.940
Moderate	12.0	08(66.7)	03(23.1)	01(8.3)		

* χ^2 Chi-square

IV. DISCUSSION

This study examined the relationship between disease severity, degree of haemolysis, and risk for stroke among hydroxyurea-naïve paediatric patients with SCA during their steady states.

The present study documented high-risk and conditional-risk TCD results in 8.0% and 21.3% of the study participants, respectively. These figures are comparable to the 6.7% and 19.7% reported by Lagunju *et al* (17) in Ibadan, also in Southwestern Nigeria, but much higher than the 3.0% and 11.0% reported by Ismail *et al* (18) in Northern Nigeria. The reason for these wide variations is not clear, but it may be suggestive of differences in the disease severity among children and adolescents with SCA in different parts of the country. Varying degrees of severity in different regions of the same country have been reported in previous studies in Saudi Arabia (19–21), with the co-inheritance of alpha-thalassaemia, specific disease haplotype and the HbF level identified as factors responsible for the observed regional differences. (19–21) The possibility of regional differences in disease expression has, however, not been explored in our practice setting.

The finding of conditional risk (CR) velocities in about 20% of subjects in this study as well as in the report by Lagunju *et al* is a cause for concern; although the stroke risk among patients with CR velocities is lower at 5%/year, as compared with 9%/year for patients with high risk velocities, (17) those with CR velocities are at risk of progressive increase in their flow velocities over time to the high risk range- especially in the absence of disease-modifying interventions such as chronic blood transfusion and the use of hydroxyurea. (17)

The highest TAMMV readings in the present study were documented in the left internal carotid (ICA) and middle cerebral arteries (MCA), which is consistent with findings in SCA subjects from a study by Lagunju (17) and her colleagues in Ibadan, Nigeria. Stroke in children with SCD

commonly results from cerebral infarction associated with occlusive vasculopathy involving the distal ICA, and proximal MCA and anterior cerebral arteries (ACA). (22) The progressive narrowing of these vessels results from fibrous intimal proliferation, which is believed to develop months or years before the onset of cerebral symptoms. A neuropathological study by Rothman *et al* (23) demonstrated cerebral infarcts in 50% of autopsied patients with SCA. These infarcts occurred most extensively in the territories supplied by the distal ICA, particularly the anterior-middle cerebral artery boundary zone, and were regularly associated with organizing and recanalizing thrombi involving the divisions of the internal carotid system.

The present study demonstrated moderate anaemia, reticulocytosis and elevated serum LDH in children and adolescents with SCA. These findings are in keeping with the chronic haemolytic state in SCA and are also consistent with findings from earlier studies. (24) None of the subjects in this study had severe anaemia, which could be the impact of routine folic acid and vitamin B12 supplementation. The majority of the subjects also belonged to the high and middle socioeconomic classes, which could have significantly impacted their nutritional status, along with the regular joint counselling sessions during clinic visits where information pertaining to nutrition, and the early recognition of sickle cell crises and other complications was regularly provided.

A significant negative correlation was observed between haematocrit levels and flow velocities in all the right-sided vessels, while there was a strong positive correlation between LDH levels and the ACA and ICA on both sides. This emphasises the relationship between haemolysis and risk for stroke as obtained from the TCD readings. Elevated serum LDH concentration has been highlighted as an important marker of haemolysis in individuals with SCA. (25) The median steady-state LDH concentration in both males and females found



in this study is comparable to mean values reported in the cohorts studied by Kato *et al.* (25)

None of the subjects in this study had severe disease; this contrasts with the observations of Adegoke and colleagues (16) in Southwestern Nigeria, in which 10.4% of their subjects had severe disease and only 33.9% had mild disease. It should be noted that the present study excluded children with established complications of SCA, such as stroke, nephropathy and hepatic complications, which could account for none of the participants having severe disease as determined by the Adegoke scoring system. (16) Additionally, high awareness of SCA in the study population, which focuses on prevention and early presentation, may have contributed to the observed severity pattern. Measures like regular clinic visits, adherence to routine medications, counselling on care, and early identification of crises and complications also play a role in maintaining lower disease severity in the SCA population.

V. CONCLUSIONS AND RECOMMENDATIONS

The present study demonstrated a relationship between markers of haemolysis, such as low steady state haematocrit and high LDH, with risk for infarctive stroke as determined by TCD in children with sickle cell disease. Despite the high burden of SCD and stroke in our practice setting, access to TCD is limited due to financial and logistical constraints. Evaluation of markers of haemolysis and assessment of disease severity could therefore be useful indicators of stroke risk in resource-poor settings where TCD may not be available or immediately feasible.

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DECLARATIONS

The authors declare no conflict of interest.

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