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Original Article

Clinical Profile and middle cerebral artery velocity of children with sickle cell anaemia seen in UUTH, Uyo, Akwa Ibom state, Nigeria.

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Abstract

Background: The risk of stroke in individuals with Sickle Cell Anaemia (SCA) can be assessed by routine non-invasive measurement of their cerebral blood flow using a Transcranial Doppler (TCD) ultrasound scan. This study aimed to determine the difference in blood flow velocity parameters in the middle cerebral artery (MCA) of children with sickle cell anaemia compared to a normal age-matched population.

Methodology: This was a hospital-based comparative cross-sectional study among 40 SCA patients aged 3-16 years, in steady state and 40 age and sex-matched HbAA healthy subjects. This study lasted from June to October 2019. Medical history was retrieved using a structured questionnaire. The time-averaged mean of maximum velocity (TAMMV) of the right and left MCA was measured using non-imaging TCD.

Results: The mean age ± SD of the SCA patients was 9.1 ± 4.4 years. The SCA patients and sex and age-matched HbAA group consisted of 23 (57.5%) males and 17 (42.5%) females respectively. SCA patients had a significantly lower mean ± SD haemoglobin (Hb) than the controls (7.1 ± 1.1g/dl vs 11.1 ± 1.4g/dl; p<0.001). The right MCA of the patients with SCA had a significantly higher mean flow velocity compared to the controls (94.1 ± 23.1 vs 55.0 ± 8.8cm/sec, p<0.001).

Conclusion: The mean TAMMV recorded in the SCA subjects were significantly higher than that of the non-SCA subjects. There is a need to ensure that TCD ultrasound is employed as a routine screening tool for stroke risk among SCA patients in Nigerian tertiary health institutions.

Keywords: Sickle Cell Anaemia; Middle Cerebral Artery Velocity; Transcranial Doppler Ultrasonography; Stroke; Children.

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Introduction

Sickle cell anaemia (SCA) is the most common form of Sickle cell disease. It is an autosomal recessive chronic haemolytic disease that is caused by homozygous inheritance of HbS denoted as haemoglobin SS (HbSS). [1] SCA is common in people of African and Mediterranean ancestry. It accounts for about 2-3% of the Nigerian population. Kaine *et al* found an HbSS prevalence of 1.6% among preschool Igbo in Eastern Nigeria. [2] Chinawa *et al*, reported 51 HbSS children in the steady state aged 3-17 years over four months in Eastern Nigeria. [3, 4] In the University of Uyo Teaching Hospital (UUTH), among all Haemoglobin genotype tests, HbSS had a prevalence of 1.5%. [5] SCA causes significant morbidity and mortality. In children particularly, it is the major cause of preventable stroke. Children with SCA carry a 300-fold increased risk for stroke, making SCA the most common cause of childhood stroke. [6] In children with SCA under the age of 10 years, the prevalence of overt stroke is 10-15%. [7] Among Nigerian children with SCA, the estimated prevalence is between 4.3% and 6.8%. Abdullahi *et al* reported an initial stroke incidence rate of 0.88 per 100 patient-years among HbSS children in northern Nigeria.[8] In addition, an estimated 40–75% of children with SCA who suffer a first stroke will have a recurrence without any intervention. [6, 9-11]

Stroke in children with HbSS is usually infarctive. The infarction is often associated with stenosis or occlusion of affected vessels most commonly the distal internal carotid, proximal middle cerebral and anterior cerebral arteries. SCD contributes to the stenosis by providing a source of persistent endothelial injury via the effects of hypoxia, increased shear stress, abnormal endothelial adherence of sickled red blood cells, and inflammation induced by reperfusion injury.[12] These trigger the production of cytokines resulting in endothelial dysregulation, upregulation of pro-inflammatory cells and activation of prothrombic and proadhesive molecules, and intracellular adhesion molecule, while inhibiting the production of cytoprotective mediators (e.g. Nitric oxide).[13] In addition, the hypoxia-induced vasodilation of SCD leads to a decrease in cerebral reserve capacity which in the presence of large vessel stenosis or systemic effects of sickled cells may further compromise perfusion to vulnerable areas of the brain resulting in distal field infarctions.[14] Haemorrhagic stroke tends to occur between 20 and 29 years of age and is associated with low steady state haemoglobin levels and results from rupture of vessels within the circle of Willis. [15]

Transcranial Doppler Ultrasonography (TCD) is a useful tool in detecting vasculopathy in children with SCA to help plan effective prevention interventions. [16] Blood flow velocity, measured using TCD is generally increased in severe anaemia in these patients, and it becomes elevated in a focal manner when stenosis reduces arterial diameter. Children with SCA who have a high risk of developing stroke can be detected with TCD ultrasonography months to years before the stroke. This is done when these patients are in a steady state which refers to a point in time when the patient is not experiencing an acute painful crisis or any changes due to therapy. Middle cerebral artery blood flow with a time-averaged mean maximum velocity (TAMMV) over 200cm/s portends a high risk for stroke. It is an indication to commence hyper-transfusion therapy as this is known to reduce stroke occurrence by about 90%. [17]

Transcranial Doppler ultrasonography can detect intracranial arterial stenosis and seems ideally suited for screening large-vessel disease in patients with SCA because it is readily available cheap, real-time characteristic, and does not employ ionizing radiation, therefore children friendly and most importantly non-invasive and well tolerated by children.[18] There are two broad types of TCD, Imaging (TCDI) and non-imaging TCD. The TCDI allows the outlining of parenchymal structures and visualization of the examined vessels, thus it has the potential of being more accurate in the estimation of the risk of stroke than the non-imaging TCD. [19] Blood flow velocities could be measured through the various acoustic windows: transtemporal, transorbital, submandibular and trans occipital routes. The transtemporal window is commonly used as it is the most accessible acoustic window. In addition, a low frequency

probe of 2 MHz is used for better penetration of the thick skull bone. Transcranial Doppler ultrasonography velocities in the middle cerebral or distal carotid artery are usually defined as normal (TAMMV <170cm/s), Borderline or conditional (170-199cm/sec) and abnormal (>200cmm/sec). [20] TCD can identify children at risk of CVA with a sensitivity of ninety per cent (90%) and a specificity of hundred per cent (100%) when compared with cerebral angiography. [21] The stroke prevention trial in SCA study (STOP) recommends routine screening with TCD ultrasonography in children aged 2-16 years with SCA with a repeat in three months for those with abnormal velocities. [22, 23] Studies from Southwestern Nigeria have reported a prevalence of 4.7% to 10.8% for abnormal TAMMV in SCA patients. [24-26]

There is a paucity of studies on TCD in the risk assessment of stroke in children with sickle cell anaemia in our environment despite the significant prevalence, morbidity and mortality from stroke in this group of children.

This study is therefore necessary to explore the middle cerebral artery velocity changes in SCA patients in this environment. This will help in identifying SCA patients who are at risk of developing cerebral vasculopathy necessitating prompt preventive intervention.

Methods

Study Population

The study involved 40 confirmed sickle cell anaemia patients aged 3-16 years in steady state who met the inclusion criteria. Steady state was defined as the absence of any crisis for at least four consecutive weeks with no history of blood transfusion in the previous four months before the screening. The comparison group were 40 Haemoglobin genotype AA (HbAA) age and sex-matched children aged 3-16 years.

Study design.

This was a comparative cross-sectional study.

Study Area

The study subjects were recruited from the sickle cell clinic and the general paediatric outpatient clinics. This study lasted for a duration of five (5) months; from June to October 2019.

Sampling Procedure

Sickle Cell Anaemia patients in steady state, whose parents gave consent were consecutively recruited until the sample size was achieved. Children with a previous CVA and those on hydroxyurea were excluded. Age and sex-matched HbAA healthy controls who came on follow-up visits and routine health checks were recruited from the general paediatric outpatient.

Demographic data (age and gender), haemoglobin genotype, current haemoglobin level, previous history of multiple blood transfusions and Doppler ultrasound findings were captured in a structured proforma.

Two ml of blood was collected to determine the Hb concentration and genotype before conducting the TCD.

Transcranial Doppler was performed by one of the authors (C.A) a trained radiologist with a high resolution real-time grey scale and Doppler ultrasound scanner TOSHIBA NemioMX®, Model SSA-590A (Toshiba medical systems corporation, Japan 2011), using a sector transducer with a frequency of 2MHz. The grey-scale scanning was done with the patient in the supine position. The patient's face was turned away from the region being scanned. Coupling gel was applied to the skin over the squamous

tempora (just above the zygomatic arch) to obliterate the air interface between the probe and the skin. The probe was gently positioned over the gel and the posterior window provided the best access to the intracranial circulation in most subjects. From this approach, the beam was directed anterosuperiorly and a large sample volume was employed. For colour flow imaging, the transducer was placed on the temporal bone either above the zygomatic arch or anterior to the external auditory canal or slightly more posterior, above the earlobe. The ipsilateral MCA was colour-coded red denoting flow towards the transducer at a depth of 40-65mm. The spectral flow also showed flow towards the transducer displayed above the zero baseline. The spectral wave tracing was done automatically by the machine or manually to obtain the TAMMV of the MCA, without angle correction or using a small Doppler angle close to zero (<60°) to the longitudinal axis of the vessel, for optimal spectral pattern. The pulse repetition frequency was set to avoid aliasing. Sampling was obtained from the right MCA and TAMMV obtained from the waveform tracing were recorded. The highest velocity in each artery was recorded as the TAMMV. TAMMV less than 170 cm/second was considered normal, values greater or equal to 170cm/sec but less than 200 cm/second were conditional risks and velocity at least 200 cm/ second was considered abnormal. [(23)]

Sample size estimation.

The study size was calculated as for a quantitative cross-sectional study, using the formula below, with a known sickle cell anaemia prevalence rate of 1.5%.[(5)]

$n = Z^2 (p(1-p)/d^2)$[(27)]

Where n= the minimum required sample size

Z = standardized normal deviation = 1.96

p = the estimate of the prevalence of the number of people with sickle cell anaemia in the population based on previous/pilot studies = 1.5% = 0.015

d = Absolute error or precision (degree of accuracy) at type 1 error set at 5% = 0.05

Hence, the sample size is calculated as:

$n= (1.96)^2 X (0.015(1-0.015)/0.05^2)$

$n = 22.7 \approx 23$

A sample size of 40 subjects each for the study and control groups were recruited to account for attrition, giving a total of 80 subjects.

Ethical considerations

Ethical approval was obtained from the institutional ethics committee. Informed consent was obtained from caregivers/parents and assent was obtained from older children.

Data management

The data was analysed with the Statistical Package for Social Science version 22.0 (SPSS Inc., IL, USA). Continuous variables such as age, haemoglobin and middle cerebral artery velocity were summarized as mean and standard deviation. Categorical variables such as gender and age group were summarized using simple frequencies and percentages. The differences in the mean of the continuous variables between the SCA patients and Controls as well as between genders were compared using the student T test. Analysis of variance (ANOVA) was used to compare the continuous variables across the age groups. Categorical variables were compared using the Chi square test or Fishers exact test as appropriate.

Data obtained from the various measurements were presented in tables, Box and Whisker's plot.

Results

A total of 80 subjects comprising 40 subjects with haemoglobin SS (SCA) and 40 non-SCA controls participated in the study.

Demographic parameter

The mean and standard deviation (mean ± SD) age for the SCA patients was 9.1 ± 4.4 years. The peak age for both SCA patients and HbAA children was in the 10 – 14 age group. (Figure 1)

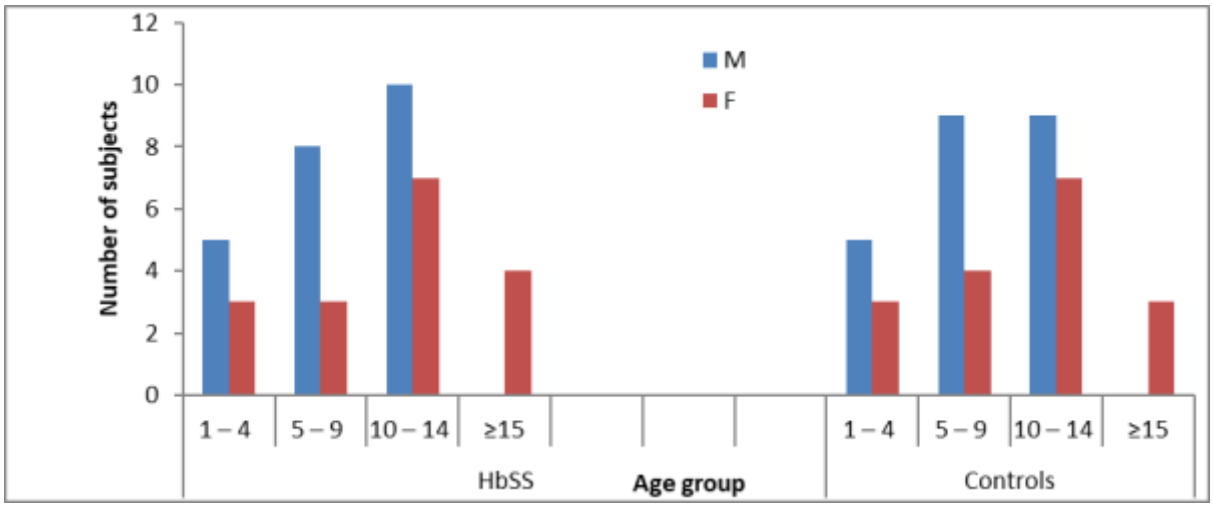

Figure 1: Age and Sex Distribution of the Study Population

Females had a higher age distribution than the males in both the subjects (10.1 ± 4.8 vs 8.4 ±4.0yrs, p = 0.226) and the controls (9.5 ± 4.5 vs 8.2 ± 3.7yrs; p = 0.303). There were however no statistically significant gender differences between the SCA patients and the controls. (male: p = 0.879; female: p = 0.743).

Table I: Age distribution by gender of the Study Population

	Male		Female		t test	*P value*
	Mean ± SD	Range	Mean ± SD	Range		
HbSS	8.4 ± **4.0**	3.0 – 14.0	10.1 ± **4.8**	3.0 – 16.0	-1.231	*0.226*
Controls	8.2 ± **3.7**	3.0 – 14.0	9.5 ± **4.5**	3.0 – 16.0	-1.045	*0.303*
t test	0.154		0.331			
P value	*0.879*		0.743			

Haemoglobin levels

The mean ± SD Hb of the SCA patients was 7.1 ± 1.1g/dl with a range of 5.6 to 10.2g/dl while that of the controls was 11.1 ± 1.4g/dl with a range of 8.0 – 13.5g/dl. The difference in mean haemoglobin levels between the SCA patients and controls was statistically significant (p <0.001). Figure 2

There was no significant difference in Hb levels between male and female SCA patients (7.0 ± 1.0 vs 7.2 ± 1.3g/dl, p =0.525). However, in the controls, male subjects had significantly higher Hb levels compared to females (11.6 ± 1.4 vs 10.4 ± 1.3g/dl, p = 0.010).

Comparing haemoglobin concentrations of SCA subjects and Controls based on sex, males and females with SCA had significantly lower haemoglobin concentrations than the Controls (p <0.001 and p <0.001 respectively).

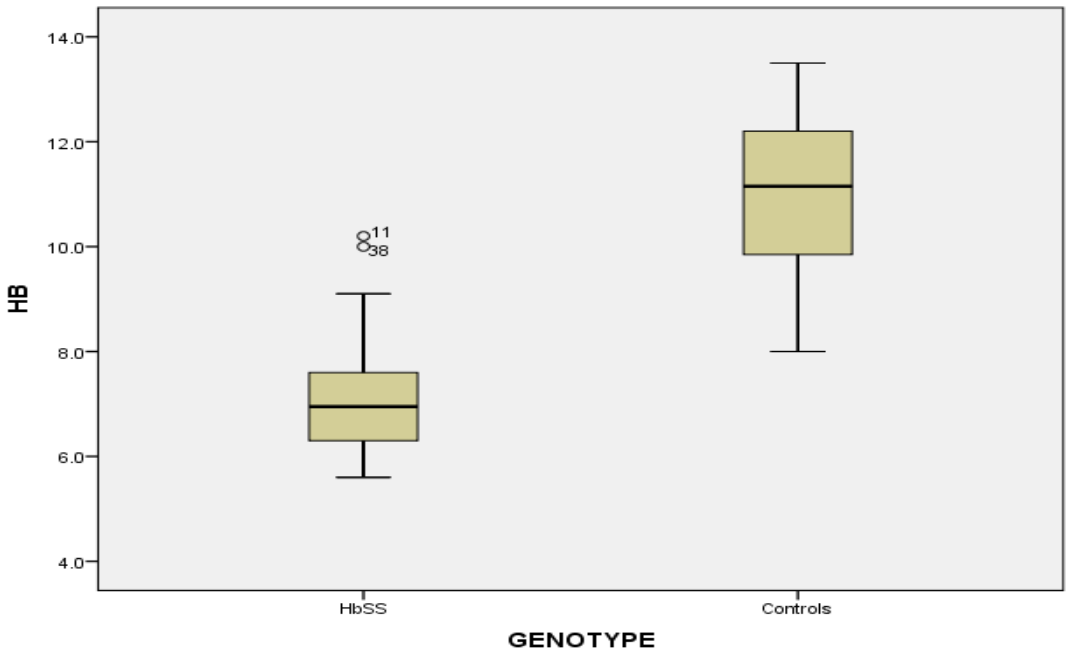

Figure 2: Haemoglobin concentration of SCA and controls.

Table II: Mean Haemoglobin concentration by Gender in Study Population

	Male		**Female**		**t test**	***P value***
	Mean ± SD	**Range**	**Mean ± SD**	**Range**		
HbSS	7.0 ± 1.0	5.8 – 10.0	7.2 ± 1.3	5.1- 10.2	-0.641	*0.525*
Controls	11.6 ± 1.4	8.9 – 13.5	10.4 ± 1.3	8.0 – 12.6	2.707	*0.010*
t test	-13.041		-7.284			
P value	<0.001		<0.001			

Right Middle Cerebral Artery Doppler Flow Velocity

Children with SCA had a significantly higher right middle cerebral artery velocity compared to the controls (94.1 ± 23.1 vs 55.0 ± 8.8cm/sec, p<0.001) with a range of 37.7 – 131.2cm/s for SCA subjects and 39.0 – 72.2cm/s for the controls. (Figure 3)

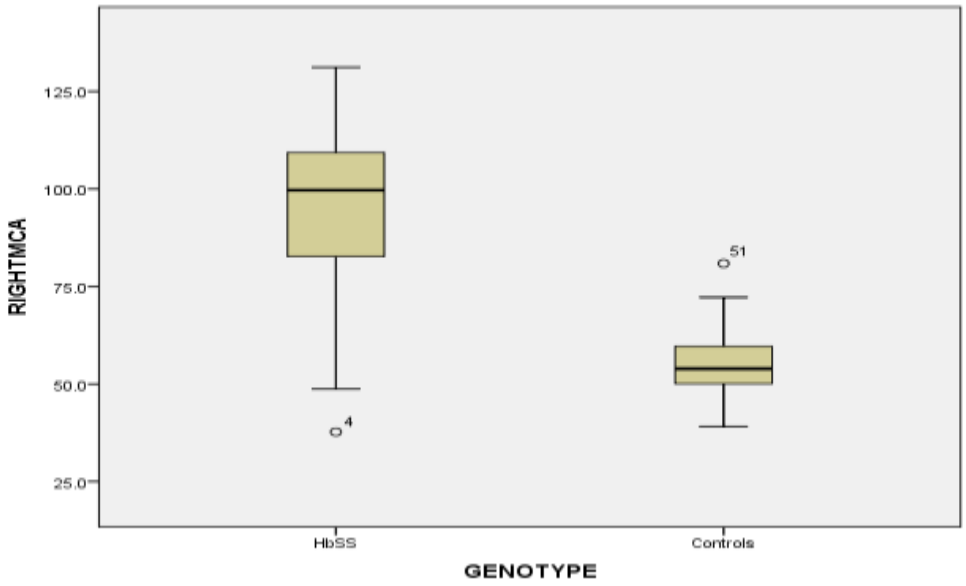

Figure 3: Box and whiskers plot of Right MCA Doppler flow velocity in Study Population

Right Middle Cerebral Artery Doppler Flow Velocity by Gender

There was no statistically significant gender difference in the right middle cerebral artery velocity in both the SCA (94.7 ± 22.3 vs 93.2 ± 25.2cm/sec; p =0.854) and the control groups (53.8 ± 9.6 vs 56.7 ± 7.5cm/sec, p = 0.307). In addition, male SCA subjects had significantly higher Right MCA Doppler flow velocity than the male Controls (p<0.001). Similarly, the female SCA had higher right MCA Doppler flow velocity than the female Controls (p<0.001).

Table III: Right MCA distribution by gender in the study population

	Male		**Female**		**t test**	***P value***
	Mean ± SD	**Range**	**Mean ± SD**	**Range**		
HbSS	94.7 ± 22.3	37.7 – 121.9	93.2 ± 25.2	48.7 – 131.2	0.185	*0.854*
Controls	53.8 ± 9.6	39.0 – 80.9	56.7 ± 7.5	48.1 – 72.2	-1.036	*0.307*
t test	8.090		5.723			
P value	<0.001		<0.001			

Discussion

Sickle cell anaemia is a common genetic disorder of the red blood cells that leads to various complications. One of the devastating complications of SCA is childhood stroke. Childhood stroke secondary to SCA can be prevented if the cerebral artery blood flow is routinely monitored and early intervention with chronic transfusion therapy is commenced in those with abnormal TAMMV.

We set out to evaluate the effect of sickle cell anaemia on the blood flow velocity within the intracranial arteries in children and to compare it to those of children with the HbAA genotype. Children in the 5–14-year-old age group constituted the largest number of subjects both in the SCA and control groups. This is similar to the study by Akinyanju where he reported that the prevalence of SCA among babies born to Nigerian parents progressively decreases through late childhood, adolescence and adulthood. [(28)] This finding may be due to the late presentation of children with SCA due to the paucity of early neonatal screening facilities in Nigeria. In addition, the early death of SCA children before adolescence and adulthood due to ignorance and the poor medical services in developing countries like Nigeria may contribute to their short lifespan.

The lack of a significant gender difference among our sickle cell participants gives additional credence to the autosomal recessive nature of its inheritance, where the affected gene is on one of the first 22 pairs of chromosomes that do not determine gender. [(29)] The absence of gender disparity has also been reported in previous Nigerian studies. [(29, 30)] We observed a significantly higher haemoglobin concentration in the control participants compared to the children with SCA (11.1 ± 1.4g/dl vs 7.1 ± 1.1g/dl; p <0.001). This finding is similar to that reported in the study of Ismail, A. *et al*, where the mean Hb concentration of 7.3g/dl was reported for SCA compared to the mean Hb concentration of 11.03g/dl in those with HbAA genotype. [(30)] In a study conducted in Sudan, Ismail MW *et al* reported a mean Hb concentration of 7.33g/dl ±1.2 among SCA children 2-18yrs. [[(16)]] This can be attributed to the increased loss of red blood cells due to chronic haemolysis, auto splenectomy and chronic inflammation in SCA patients compared to normal subjects.

We did not observe any significant gender difference in the Hb concentration of children with SCA (7.0 ± 1.0 v7.2 ± 1.3; p=0.525). However, there was a significant gender difference in the Hb Concentration of the control group with the males having a higher Hb concentration than the female subjects (11.6 ± 1.4 vs 10.4 ± 1.3: p=0.01). This may be due to the stimulatory effect of androgen secretion especially in adolescent males and blood loss during menstrual cycles in adolescent females some of whom were part of this study. This hormonal effect is likely not prominent in children with SCA since they have a later onset of puberty compared to children with the HbAA genotype.[(31)]

This study observed that the mean blood velocities in MCAs of SCA patients were significantly higher than those of the controls ((91.1 ±23.1cm/sec vs 55.0 ±8.8cm/sec; p<0.001). This can be explained by the brain vascular response to mitigate the chronic hypoxia as a result of the chronic anaemic status. [(28)] Lagunju *et al* using non-imaging TCD among 145 Nigerian children with SCA recorded a mean TAMMV of 152± 27.0cm/s which was significantly higher than the mean of that recorded in this study. [(24)] The longitudinal nature of the study by Lagunju *et al* that allowed for serial measurements and the possibilities of conversion from one TAMMV risk category to another may have contributed to the differences in both studies. In addition, the observation that most of our study population were in the 10-14 age group may also account for the low mean TAMMV in our study. This was corroborated by Adekunle *et al* who observed that the mean total TAMMV was highest in the below-five age group and lowest in the subjects above ten years with a value of 161±26 and 149±31cm/sec respectively.[(26)]

The index study observed similar mean TAMMV values in males and females both in the SCA subjects and controls. This is similar to what was reported in the study by Ismail A. *et al* who found no significant difference in right MCA TAMMV between sexes (p-value 0.416). [(30)] The reason for this is unclear. Larger studies are needed to ascertain any disparity.

Based on the STOP trial criteria, The TAMMV is used to categorise patients into three risk groups [32] (1) Normal velocities (standard risk) < 170cm/s; this confers a 2% risk of CVA to patients. (2) Conditional velocities (intermediate risk) 170 to199cm/s; carrying 7% risk of stroke. (3) Abnormal velocities (High risk) ≥200m/s; conferring 40% risk of stroke to sufferers.[22] In the index study, there was no SCA subject within the high-risk group. This was probably due to the stringent inclusion criteria that eliminated most of the high-risk SCA subjects who weren't in a steady state. Also, most of the subjects who participated in this study had good health-seeking behaviour as they kept appointments with their doctors and adhered to medications. This finding is similar to that seen at El-Obeid Children Hospital by Ismail M. *et al*, where no SCA subject was found to be within the high-risk group. [16] A Kenyan study of 105 children with SCA also found a 0% prevalence of high-risk TCD velocities. [33] The STOP Trial recorded a prevalence of 9.3% high-risk children categories. [22] Lagunju *et al* reported a prevalence of 4.7% with TAMMV ≥200mls among children with HbSS. [24] Adekunle *et al* also recorded a 10.8% prevalence in the high-risk category.[(26)] However, other studies elsewhere in North America observed subjects in all three categories. [22] Differences in sample size and study designs may be responsible for these disparities in categorising risk groups. However, extensive studies with large sample sizes are required to address these disparities.

Limitations

Transcranial Doppler ultrasonography is operator-dependent, therefore intra-observer variations in measurements may have occurred, but this was minimised by taking an average of three measurements. The small sample size which was necessitated by the self-funded nature of the study may have contributed to some of the disparities observed with studies with larger sample sizes.

Conclusion

This study of sickle cell anaemia children in South-south Nigeria has shown that there is a lower prevalence of elevated TCD velocities compared to studies done in the northern and western parts of the country as well as those done outside Nigeria. We, therefore, recommend the need for larger nationwide studies to establish more appropriate reference ranges for TCD velocities in Nigerian children with SCA. In addition, there is also a need to ensure that TCD ultrasound is employed as a routine screening tool for stroke risk among SCA patients in Nigerian tertiary health institutions.

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