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Jibir Binta Wudil
Wudil J. Usman
Muuta I
Adeodu O. Oyewole

Assessment of the risk of stroke in children with sickle cell anemia using transcranial doppler ultrasound with imaging in Northwestern Nigeria

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Jibir Binta Wudil (✉)
Khalifa Sheik Isyaka Rabiu
Paeditric Hospita,
Zoo Road, Kano, Nigeria
Email: bintajibir@gmail.com

Wudil J. Usman
Vanderbilt Institute for Global Health, Vanderbilt University Medical Center, Nashville, TN, USA.

Muuta I
Aminu Kano Teaching Hospital/ Bayero University, Kano, Nigeria.

Adeodu O. Oyewole
Obafemi Awolowo University, Ile-Ife, Osun, Nigeria.

Abstract: *Background:* Stroke is a major cause of morbidity and mortality affecting about 10% of patients with sickle cell anemia (SCA). Transcranial Doppler (TCD) ultrasonography helps to identify children with SCA who are at an increased risk for stroke. This study aimed to determine the risk of stroke in children with SCA in steady state using Transcranial Doppler with Imaging (TCDI) scan abnormalities and the prevalence of TCDI scan abnormalities among children with SCA in northern Nigeria.
Materials and Methods: We conducted acomparative study among 240 persons with SCA who attended a pediatric sickle cell clinic in northern Nigeria and were in steady state condition. We performed a transcranial ultrasound examination and collected blood samples to perform full blood counts and hemoglobin electrophoresis.
Results: Abnormal velocities were obtained in 11 (9.3%) children with SCA; while 16 (13.6%) had conditional velocities and 89 (76.7%) of those had normal velocities in one or more of the four vessels studied. Most of the children in the control group 116 (96.7%) had normal velocities and the difference between the groups was not statistically significant ($\chi^2 = 0.59$, $p > 0.05$).
Conclusion: The use of TCDI sonography to predict the risk of stroke should be included in the standard of care in children with SCA in Nigeria and should be included in routine evaluation of disease severity in children with SCA

Key Words: Transcranial Doppler Imaging; sickle cell anemia; stroke in children.

Introduction

Sickle Cell Disease (SCD) is one of the most common inherited disorders in the world. It is estimated that over three quarters of the world's three million people with SCD live in Sub-Saharan Africa, with the highest prevalence being in Nigeria where about 150,000 children are born annually with SCD giving a prevalence rate of 20 per 1000 births.[1]

SCD often results from the inheritance of two sickle cell (S) mutant genes or from the inheritance of one S gene mutation with another hemoglobin; C gene (sickle cell hemoglobin C disease), β-thalassemia gene (sickle-thalassemia disease), or another β-globin variant. People homozygous for the HbS gene (HbSS) are said to have sickle cell anemia (SCA). Certain complications may be common, depending on the subtype. In addition, β-haplotypes may influence the phenotype. On average, patients with hemoglobin C or α-thalassemia and most patients with β-thalassemia have milder neurological complications than those with SCA.[2]

Stroke is a major cause of morbidity and mortality in SCD affecting about 10% of patients with homozygous SCD,[7,8] a prevalence that is 250 times greater than in the general pediatric population.[3-8] Overt stroke is a focal neurologic deficit resulting from cerebrovascular compromise that persists for more than 24 hours and has neuroimaging evidence of a cerebral infarct corresponding to the focal deficit. In addition, subclinical cerebrovascular disease or silent infarcts, identifiable by magnetic resonance imaging studies, are present in another 10% to 20% of patients.[6,7] Children with SCA are at high risk of stroke and about 11% have at least one episode by 11 years of age.[8] The estimated prevalence of stroke in children with SCA in Nigeria is 5–7%.[9-11] The risk is lowest before the age of two years, probably because of the protective influence of fetal hemoglobin on sickling.[8]

Both overt and silent strokes in sickle cell anemia (SCA) are associated with significant cognitive deficits.[6,7] In addition acute strokes and chronic cerebral ischemia are among the most disabling severe consequences of sickle cell disease.[12] The fragile cerebral vessels of young children are particularly vulnerable and crippling strokes may blight a child's life even before the age of two years.

Transcranial Doppler (TCD) ultrasonography helps to identify children with SCD who are at an increased risk for stroke.[8] TCD examinations are low cost and free of side effects. The use of TCD in clinical research in SCA is justified by the disease's pathophysiology, which involves the great ce- rebral vessels, which can be very well assessed by TCD.
TCD recording of cerebral blood flow can be done using non-duplex, non-imaging ultrasound machine (TCD) or the use of duplex imaging ultrasonography, otherwise called transcranial Doppler imaging (TCDI) which uses the more widely available ultrasound machine. TCDI is easier to learn and use and with few adjustments can provide equivalent predictive power to non-imaging TCD measurements. It therefore offers an opportunity to apply an effective therapy for patients in this risk group and reduce many first-time strokes.[12,13]

TCDI is however underutilized in the West African Sub-Region as a clinical tool despite well-established indications. Based on extensive literature search on Google Scholar this study is the first in Nigeria that used TCDI to assess the risk of stroke in children with SCA. This may be due in part to lack of awareness about its diagnostic usefulness. Most studies from West Africa and other parts of the world use the more conventional non imaging TCD to evaluate the risk of stroke. Therefore, this study aimed to determine the risk of stroke in children with SCA in steady state using Transcranial Doppler ultrasound with imaging, to determine the prevalence of TCDI scan abnormalities among children with SCA in steady state, and also determine the relationship between the prevalence of TCDI abnormalities and the clinical severity of SCA among children with SCA in children with sickle cell anemia in northern Nigeria.

Materials and Methods

Setting

The study was conducted at Murtala Muhammed Specialist Hospital (MMSH), astate-owned tertiary health institution in Kano State. The Pediatric Sickle Cell Clinic unit provides services for patients aged 6 months to 16 years and was launched in 1980. As of November 2012, the clinic had a total of 11,000 patients registered, with an average daily clinic attendance of 70 to 100 SCD patients.[14]

Study Design

This was a comparative study of children with SCA in steady state and attended the SCD Pediatric Clinic of Murtala Muhammad Specialist Hospital (MMSH), and was conducted between August 2013 and June 2014 (10 months). Informed consent (signed or thumb printed) and assent were obtained from caregivers and older children (≥7years) respectively. Children who met the inclusion criteria were enrolled for the study. The inclusion and exclusion criteria are listed below:

Inclusion Criteria

1. Children with SCA in steady state, aged 2 to 16 years.

Exclusion Criteria

1. Children that have had a cerebrovascular event with symptoms lasting more than 24 hours.[(15)]
2. Children on hydroxyurea therapy, medications like extract of Fajara Zanthoxyloides (Nicosan) and Resveratrol, or patients with a history of bone marrow or stem cell transplant.
3. Children with history of chronic diseases e.g., diabetes mellitus, chronic kidney disease and AIDS
4. Children with a history of blood transfusion within the preceding 3 months

Steady state was defined as that period free of crisis extending from at least four weeks since the last clinical event and three months or more since the last transfusion of blood or blood products, to at least one week before the start of a new clinical event.[16,17]

Sample Size Determination and sampling technique

The sample size was determined using Fisher's formula for calculating minimum sample size for descriptive studies, i.e., $n = z^2pq/d^2$, where:
n = minimum sample size
z = percentage point of standard normal distribution curve which corresponds to 95% confidence interval.
p = prevalence rate from previous study.
q = complimentary probability; q = 1-p.
d = degree of precision at 95% confidence limit;
d = 5% = 0.05
By substituting these values into the formula, and using a prevalence of 7% of stroke among patients with SCD.[1,8,18]

$n = 1.96^2 \times 0.07 \times 0.93/0.05^2 = 100$.

The calculated sample size was adjusted upwards by 20% to account for incomplete responses and those with inadequate TCD results. Thus 120 Subjects and 120 Controls were enrolled.[(19)]Systematic sampling method was used to select subjects for the study. Using a sampling interval of 12 obtained by dividing the monthly attendance at the SCD clinic (1400) with the required sample size (120), every 1 in 12 patients with SCA, aged 2 – 16 years was selected initially but subsequently

dropped if he/she failed to meet the inclusion criteria and replaced with the next patient in the sequence, until the required sample size was obtained. Similarly, 120 children with hemoglobin AA matched for age and sex, who attended the General Pediatrics Outpatient Clinic, were enrolled as Controls. Any control found to have any genotype other than HbAA by hemoglobin electrophoresis was replaced.

Study Procedure

Ultrasound examination of a vessel by means of Doppler scan including TCD is referred to as insonation. TCDI evaluations were performed in a clinic setting by the principal investigator and a trained radiologist using PSM-30 BT Toshiba (Tokyo, Japan).
The protocol recommended use of a 4–6-mm sample volume size, spectral display at 250 cm/s, and focal depth adjusted close to 8 cm to view the midline.[20] The middle cerebral artery (MCA) and intracranial internal carotid artery (ICA) were insonated with the transducer over the temporal bone just anterior to the ear. Adequate insonation of MCA and ICA arterial segments on both sides were achieved by the combination of consistent angulations at 0^0, and changes in the shape of sample volume thus giving the maximum Doppler value. The time-averaged mean of the maximum velocity (TAMMX) of the MCA and ICA flow on Doppler spectrum were also estimated. The results obtained were classified as either normal or abnormal based on the highest velocity of flow in the MCA. Based on previous studies abnormal velocity was defined as TAMMX of ≥ 180cm/sec and conditional velocity as155–179 cm/sec.[21-23] Blood samples were obtained for full blood count and hemoglobin electrophoresis (Controls only), using cellulose acetate paper at pH of 8.6.

Data collection

Pro-forma for recording biodata, history and physical examination findings, results of laboratory investigation and TCDI velocities were used to collect data.

Data Analysis

Data validation and analysis were done using Statistical Package for Social Sciences (SPSS®) version 20 (Chicago, IL. USA) statistical software. Qualitative data were summarized using frequencies and percentages, while quantitative data were summarized using mean and standard deviation. Nutritional status was assessed using the Z scores of weight-for- age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) with reference values adapted from WHO Growth standards for children up to 5 years and WHO Growth Reference 2007 for older children.[24]An age interval that classified the children into preschool age (2-5 years), school age (6-10 years) and adolescent (11-16 years) was chosen. Analysis of variance (ANOVA) was used to analyze the differences between group means. Correlation between average MCA flow velocities and physiological parameters crisis were calculated using Pearson correlation coefficient. The Student's 't'- test was used to compare means of cerebral blood flow parameters, hematocrit values, hemoglobin; platelets and total white cell counts between the Subjects and the Control groups. A p-value of < 0.05 was considered significant.

Ethics

Ethical approval was obtained from the Ethics Committee of the Kano State Hospitals Management Board, Kano (HMB/GEN/48811). Study procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000).

Results

A total of 240 participants, 120 Subjects and 120 age and sex matched controls were recruited for the study from August 2013 to June 2014.

Age and Sex Distribution

The age and sex distribution of the Subjects and Controls is shown in table I. There were no significant differences between the subjects and controls in age and sex ($\chi^2 = 3.75$, df = 2.0, p = 0.153).

Table 1: Age and Sex distribution of Subjects and Controls

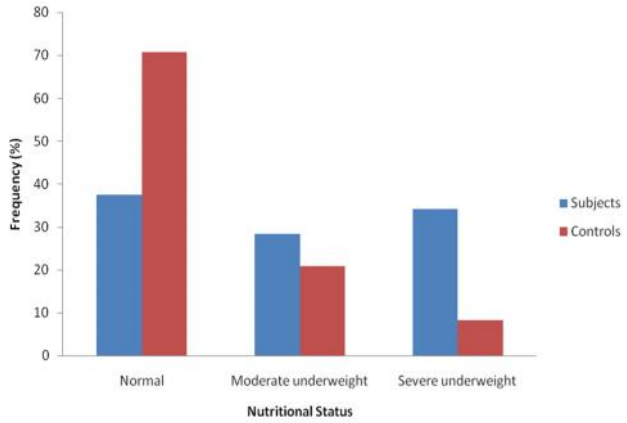
Age (years)	Subjects Male	Female	Total	Controls Male	Female	Total
2 – 5	17 (14.2)	13 (10.8)	30 (25)	17 (14.2)	13 (10.8)	30 (25)
6 – 10	17 (14.2)	28 (23.3)	45 (37.5)	17 (14.2)	28 (23.3)	45 (37.5)
11 – 16	25 (20.8)	20 (16.7)	45 (37.5)	25 (20.8)	20 (16.7)	45 (37.5)
Total	59 (49.2)	61 (50.8)	120 (100)	59 (49.2)	61 (50.8)	120 (100)
$\chi^2 = 3.75$, p = 0.153	df = 2.0					

Figures in parenthesis are percentages

Nutritional Status of Subjects and Controls

Weight-for-Age Z scores (WAZ):Overall, 110 (45.8%) of the participants were moderately to severely underweight with the subjects (mean WAZ = -2.60 ± 1.53) being more underweight than the Controls (mean WAZ = -1.24 ± 1.23). The difference in the means was statistically significant (t=7.59, p<0.001). Figure 1 shows that, among the Subjects, 37.5% had normal WAZ scores, 28.3% were moderately underweight and 34.2% were severely underweight. The controls were more adequately nourished with adequate, moderate and severe WAZ scores of 70.8%, 20.8% and 8.3% respectively.

Fig 1: Distribution of Subjects and Controls by Nutritional Status using WAZ scores



Mean Hematological parameters

The mean hemoglobin concentration and hematocrit were significantly lower in the Subjects (t=27.2, p<0.001, for the difference in mean hemoglobin and, t=27.4, p<0.001for the difference in hematocrit). Total white cell and platelets counts were significantly higher in the Subjects than Controls (t=16.6, p <0.001 for the difference in mean WBC and, t=4.6, p<0.001for the difference in mean platelet count, Table 2).

Table 2: Mean Hematological parameters of Subjects and Controls

Hematologic Parameters	No.	Mean ± SD Subjects	Controls	t-value	p-value
Hemoglobin (g/dl)	120	7.6 ± 1.3	12.0 ± 1.2	27.2	<<0.001
Hematocrit (%)	120	23.1± 4.0	37.4 ± 4.1	27.4	<<0.001
White cell count (10^9/L)	120	14.3± 4.4	6.9 ± 2.1	16.6	<<0.001
Platelet count (10^9/L)	120	386.8 ± 152.6	311 ± 95.1	4.6	<0.001

95% level of significance

Time- averaged mean of the maximum velocity (TAMMX) in middle cerebral and internal carotid arteries of Subjects and Controls

The TAMMX ranged from 41.2 to 251.8cm/sec in the Subjects and from 40.6 to 137.8 in the Control group. The means of the four major intracranial arteries of the Subjects and Controls are shown in table III. Mean TAMMX was significantly higher in the right and left middle cerebral arteries than in the internal carotid arteries. Mean TAMMX was significantly higher in all the four vessels of the subjects (t=9.9, p<0.001; t=9.1, p<0.001).

Correlation between TCDI velocities and physiological variables

The correlation coefficient and p-values for the correlations are shown in Table IV. There was a statistically significant negative correlation between steady-state hematocrit and platelet count, and a positive correlation with clinical severity of SCA and TAMMX among the Subjects. Among the Controls, there was a significant correlation between TAMMX and WBC count, age and nutritional status but not with hematocrit and platelet count.

Table 4: Correlation between TCDI velocities and Physiological variables

Variables	Subjects r	p-value	Controls r	p-value
Steady-state PCV	-0.41	<0.001	-0.17	0.073
WBC count	0.16	0.078	0.20	0.033
Platelet count	0.22	0.023	0.15	0.112
Age	-0.16	0.191	-0.54	<0.001
Clinical severity	0.33	<0.001		
Nutritional Status	-0.13	0.162	-0.25	0.007

r = correlation coefficient, PCV = Packed Cell Volume, WBC = White Blood Cells, 95% level ofsignificance

Table 3: Mean TAMMX in 2 Major intracranial Arteries in Subjects and Controls

Artery	Mean ± SD TAMMX (cm/s) Subjects N	Mean ± SD	Controls N	Mean ± SD	t-value	p-value
Right MCA	117	115.8 ± 31.7	116	83.4 ± 15.2	9.9	<0.001
Left MCA	118	116.6 ± 33.4	116	85.7 ± 15.1	9.1	<0.001
Right ICA	116	84.0 ± 21.6	116	66.4 ± 9.8	8.0	<0.001
Left ICA	117	81.7 ± 19.3	116	65.5 ± 10.6	7.9	<0.001
ANOVA: F = 54.41, p <0.001			F = 35.68, p <0.001			

Table 5: TCDI Risk Classification in two major Intracranial Arteries in the Subjects

TCDI Risk Classification	Number (%) Right MCA	Left MCA	Right ICA	Left ICA	Total
Abnormal	6 (5.1)	5 (4.2)	0	0	11 (2.4)
Conditional	4 (3.4)	11 (9.3)	1 (0.9)	0	16 (3.4)
Normal	107 (91.5)	102 (86.4)	115 (99.1)	117 (100)	441 (94.2)
Total	117 (100)	118 (100)	116 (100)	117 (100)	468 (100)

TCDI Risk Classification in 2 major intracranial arteries

The abnormalities in 464 TAMMX measurements in the Subjects are shown in Table 5. Abnormal velocities were obtained in 11 (9.3%) children in the SCA group while 16 (13.6%) had conditional velocities and 89 (76.7%) of them had normal velocities in one or more of the four vessels studied. Most of the children in the control group, 116 (96.7%) out of 120, had normal velocities. This difference was not statistically significant (χ^2 = 0.59, p > 0.05). TAMMX was abnormal (N=11) or conditional (N=16) in 27 of 468 (5.8%) measurements among the Subjects and 0 of 468 measurements among the Controls. The difference was highly statistically significant (χ^2 =27.57, p=<<0.001).The procedure was unsuccessful in 12 of 480 (2.5%) measurements (RMCA3, LMCA 2, RICA 4, and LICA 3) among the Subjects and 16 of 480 (3.3%) measurements (4 for each of the arteries) for the controls.

Discussion

This study showed that children with SCA were more significantly malnourished than their age and sex-matched controls, which is consistent with similar studies in the West African sub-region and beyond.[17,25-28] Previous studies, except for a few such as that reported by Oredugba and Savage from Nigeria have observed significant impairment in the weight and height of children with SCA.[27,28] This is attributed to the effects of chronic anemia, recurrent infections and ill-health on growth.[28] Eboyomi*et al* in Nigeria found SCD children to be below the 50th centile of their peers.[28] Rodrigues and colleagues in 2011 revealed that for all nutritional classes, there were a greater proportion of children with HbAA in the normal range than children with SCD.[26] The prevalence of malnutrition was 61.3% among SCD Subjects and 28.6% among Controls (p<0.001) among Ghanaian children with a mean age of 7.18 for Subjects and 5.13 for the Controls.[26] Interestingly, however a recent study in Nigeria showed that obesity does occur in children with SCA with an overall prevalence of 2.5%.[29] All the obese SCA children in this study were from families of high socio-economic status.[29]

The mean steady state total WBC count and platelet counts recorded in this study were significantly higher among the children with SCA than in the HbAA controls. This is similar to findings by other authors.[30-32] Leukocytosis is a risk factor for early SCD-related death, clinically overt strokes, silent cerebral infarction and acute chest syndrome.[8,33-36] However, unlike the clear effect of anemia and leukocytosis, the clinical effects of thrombocytosis on the course of SCD are not well defined, although an association between stroke and platelet count > 450,000/µl has been reported.[33,35] In addition, the increased concentration of soluble CD40 ligand (sCD40L) in the plasma of patients with SCD may be related to thrombocytosis.[(37)]SolubleCD40L could be relevant to the pathogenesis of vaso-occlusion and therefore CVA in SCA.[38]

The time-averaged means of the maximum velocity (TAMMX) in all the arteries insonated in this study was significantly higher in SCA patients than in HbAA controls. This is similar to the findings of Brass *et al* and Brambilla *et al*.[39,40] The mean MCA and mean ICA velocities in this study are lower than those reported by Adams *et al*.and Lagunju*et al*. but comparable to those reported by Hyder *et al*., whereas both Adams *et al* and Lagunju *et al*. used non-imaging TCD.[(41-44)] The difference in methodology could explain the difference in findings. The observed differences in mean velocities between the MCA and ICA are in keeping with previous reports.[22,42]

Studies have shown that TAMMX measurements are more often successfully obtained using non-imaging TCD than by TCDI, especially from the distal internal carotid artery and the anterior cerebral artery.[21,22,46] This is probably because of limitations in acquiring an adequate angle of insonation with the bulkier TCDI transducer. However, a less clear differential was reported by Neish *et al*., who used the combined imaging results from two TCDI machines (Acuson Sequoia; Logics, GE Healthcare) in comparison with a Nicolet Companion TCD machine to measure TAMMX and peak systolic velocity.[23]

Velocity is generally increased by severe anemia in patients with SCD, and it becomes elevated in a focal manner when stenosis reduces the arterial diameter. Children with SCD who are developing elevated risk for stroke can be detected months to years before the stroke using TCD. The 9.3% prevalence of abnormal velocities in SCA in this study is similar to the 8% and 9% respectively reported from the African-American cohort and the Stroke Prevention Trial (STOP) but higher than the prevalence of 4.7% reported by Lagunju *et al*.[18,43,47] However, a higher prevalence of 8.4% was reported in another recent study by Lagunju *et al*.[48] This is probably due to the repeated TCD examinations performed in that study, which allowed for the detection of more children with abnormal TCD velocities. The 13.6% prevalence of conditional velocity is lower than the 19.7% reported earlier by Lagunju *et al* from Nigeria, 15% from the STOP trial and 17.5% reported from the African American cohort.[18,43,47]

In the current study, 3.3% of children with SCA and 3.3% of Controls, had velocities that were inadequate in one or more of the vessels insonated. This is similar to the prevalence of 3 to 5% reported by Fujioka *et al*.[20] The STOP trial reported a higher inadequate velocity of 6% while Telfer reported a 5% inadequate velocity in the East London cohort.[46] However, no inadequate velocities were reported by Lagunju *et al*. Inadequate velocities arise because sonographic interrogation of part or all the basal arterial circulation is made difficult or impossible by increased calvarial thickness or because

of unusual vessel position, displacement or tortuosity.[43,50]

There was a significant negative correlation between the TAMMX and steady-state hematocrit among the SCA patients in this study, but the correlation between the TAMMX and hematocrit on enrollment was not significant among the Controls. These are similar to findings by Lagunju *et al*., Brass *et al*. and Bernaudin *et al*.[40,43,51] The correlation of TAMMX with age in HbAA children agrees with previous reports.[21,40,48,49] Even though it did not attain statistical significance, the trend in children with SCA in this study is also in keeping with previous reports.[21,43,51,52] Among the children with SCA, 55% and 44% with abnormal and conditional risk velocities were in the 2–5-year-old age bracket. This will suggest that children with SCA in the first 5 years of life should be given priority for routine TCD in low-resource settings where routine TCD for all children is not achievable. In healthy children, flow velocities in the MCA and ICA are lower in older children, probably as a result of the proportional growth of both arteries along with body development.[53-55]

The repeated TCDI examinations in this study allowed for the detection of more children with abnormal velocities, which is similar to findings from an earlier study.[48] It is therefore imperative that children with SCA and conditional velocities are followed up with repeated TCDI examinations to allow for early detection of abnormal velocities and prompt institution of stroke prevention measures.

Conclusions

The use of TCDI sonography to predict the risk of stroke should become the standard of care in children with SCA in Nigeria and should be included in the routine evaluation of disease severity in children with SCA to enhance the quality of care and management outcomes for this chronic and debilitating disease. There is also a need for further research to determine if there are other risk factors for high cerebral blood flow velocities, and a large clinical trial to determine whether hydroxyurea can lower TCDI velocities in African children. The correlation of abnormal TAMMX and MRA/MRI is also recommended as a research tool to further narrow the gap in knowledge of the consequences of TAMMX abnormalities in SCD in Sub-Saharan Africa.

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Author contributions

JBW conceptualized the study, analyzed, and interpreted the data. JBW and WUJ contributed to writing up the manuscript. MI and AOO revised the manuscript. All authors reviewed the manuscript and approved the final version for submission.

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References

1 World Health Organization. Sickle-cell anaemia: Report by the Secretariat 59th World Health Assembly. *Provisional agenda item11.4; A59//9. April 2006.*

2. Powars D, Wilson B, Imbus C, Pegelow C, *Allen J. The natural history of stroke in sickle cell disease. Am J Med 1978; 9:461-471.*

3 Earley CJ, Kittner SJ, Feeser BR, Gardner J, Epstein A, Wozniak MA, et al. Stroke in children and sickle-cell disease: Baltimore-Washington Cooperative Young Stroke Study. *Neurology 1998; 51:169-176.*

4 Balkaran B, Char G, Morris JS, Thomas PW, Serjeant BE, Serjeant GR. Stroke in a cohort of patients with homozygous sickle cell disease. *J Pediatr 1992;120:360-366.*

5 Pavlakis SG, Bello J, Prohovnik I, Sutton M, Ince C, Mohr JP, et al. Brain infarction in sickle cell anemia: Magnetic resonance imaging correlates. *Ann Neurol 1988;23:125-130.*

6 Armstrong FD, Thompson RJ, Wang W, Zimmerman R, Pegelow CH, Miller S, et al. Cognitive functioning and brain magnetic resonance imaging in children with sickle Cell disease. Neuropsychology Committee of the Cooperative Study of Sickle Cell Disease. *Pediatrics 1996;97:864–870.*

7 Craft S, Schatz J, Glauser TA, Lee B, DeBaun MR. Neuropsychologic effects of stroke in children with sickle cell anemia. *J Pediatr 1993;123:712-717.*

8 Ohene-Frempong K, Weiner SJ, Sleeper LA, Miller ST, Embury S, Moohr JW, et al. Cerebrovascular accidents in sickle cell disease: *rates and risk factors. Blood 1998;91:288–294.*

9 Oniyangi O, Ahmed P, Otuneye O, Okon J, Aikhionbare H, Olatunji O, et al. Strokes in children with sickle cell disease at the National Hospital Abuja Nigeria. *Nigerian J Paediatrics 2013;40:158-164.*

9 Lagunju I, Sodeinde O, Telfer P, Brown B. Transcranial doppler ultrasonography in primary stroke prevention in children with sickle cell disease: our initial experience: Abstract G125 Table 1 TCD screening results classified according to Adam's criteria. *Arch Dis Child 2010;95:A63.3-A64.*

11 Fatunde OJ, Adamson FG, Ogunseyinde O, Sodeinde O, Familusi JB. Stroke in Nigerian children with sickle cell disease. *Afr J Med Med Sci 2005;34:157–160.*

12 Verduzco LA, Nathan DG. Sickle cell disease and stroke. Blood 2009;114:5117-5125.

13 Switzer JA, Hess DC, Nichols FT, Adams RJ. Pathophysiology and treatment of stroke in sickle-cell disease: present and future. *Lancet Neurology 2006;5:501-512.*

14 Galadanci N, Wudil BJ, Balogun TM, Ogunrinde GO, Akinsulie A, Hasan-Hanga F, et al. Current sickle cell disease management practices in Nigeria. *Int Health 2014;6:23–28.*

15 The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. *J Clin Epidemiol 1988;41:105-114.*

16 Nebor D, Bowers A, Hardy-Dessources M-, Knight-Madden J, Romana M, Reid H, et al. Frequency of pain crises in sickle cell anemia and its relationship with the sympatho -vagal balance, blood viscosity and inflammation. *Haematologica 2011;96:1589-1594.*

17 Akinola NO, Stevens SM, Franklin IM, Nash GB, Stuart J. Subclinical ischaemic episodes during the steady state of sickle cell anaemia. *J Clin Pathol 1992;45:902-906.*

18 Adams RJ, McKie VC, Brambilla D, Carl E, Gallagher D, Nichols FT, et al. Stroke Prevention Trial in Sickle Cell Anemia. *Control Clin Trials 1998;19:110-129.*

19 Lachenbruch PA, Lwanga SK, Lemeshow S. Sample Size Determination in Health Studies: A Practical Manual. *J. American Statistical Association 1991;86:1149.*

20 Fujioka K, Douville CM. Anatomy and freehand examination techniques. In Newell DW, Aaslid R, eds. Transcranial Doppler. *New York: Raven Press, 1992; 9-31.*

21 McCarville MB, Li C, Xiong X, Wang W. Comparison of Transcranial Doppler Sonography With and Without Imaging in the Evaluation of Children With Sickle Cell Anemia. *Am J Roentgenol 2004;183:1117-1122.*

22 Bulas DI, Jones AM, Seibert JJ, Driscoll C, O'Donnell R, Adams RJ. Transcranial Doppler (TCD) screening for stroke prevention in sickle cell anemia: pitfalls in technique variation. *Pediatr Radiol 2000;30:733-738.*

23 Neish AS, Blews DE, Simms CA, Merritt RK, Spinks AJ. Screening for Stroke in Sickle Cell Anemia: Comparison of Transcranial Doppler Imaging and Nonimaging US Techniques. *Radiology 2002;222:709-714.*

24 The World Health Organization Growth reference data for 5-19 years. 2007. https://www.who.int/tools/growth-reference-data.

25 Adams RJ. TCD in sickle cell disease: an important and useful test. *Pediatr Radiol 2005;35:229–234.*

26 Osei-Yeboah C, Rodrigues O, Enweronu-Laryea C. Nutritional status of children with sickle cell disease at Korle Bu Teaching Hospital, Accra, Ghana. *West Afr J Med 2011;30:262–267.*

27 Oredugba FA, Savage KO. Anthropometric finding in Nigerian children with sickle cell disease. *Pediatr Dent 2002;24:321–325.*

28 Ebomoyi E, Adedoyin MA, Ogunlesi FO. A comparative study of the growth status of children with and without SS disease at Ilorin, Kwara State, Nigeria. *Afr J Med Med Sci 1989;18:69–74.*

29 Akodu SO, Diaku-Akinwumi IN, Njokanma OF. Obesity—Does It Occur in Nigerian Children with Sickle Cell Anemia. *Pediatr Hematol Oncol 2012;29:358-364.*

30 Awogu AU. Leucocyte counts in children with sickle cell anaemia usefulness of stable state values during infections. *West Afr J Med 2000;1:55-8.*

31 Antwi-Boasiako C, Ekem I, Abdul-Rahman M, Sey F, Doku A, Dzudzor B et al. Hematological parameters in Ghanian sickle cell disease patients. *J Blood Med 2018; 9: 203-209.*

32 Ahmed AE, Ali YZ, Al-Suliman AM, Albagshi JM, Al Salamah M, Elsayid M. The prevalence of abnormal leukocyte count, and its predisposing factors, in patients with sickle cell disease in Saudi Arabia. *J Blood Med 2017; 8:185-191.*

33 Miller ST, Sleeper LA, Pegelow CH, Enos LE, Wang WC, Weiner SJ, et al. Prediction of Adverse Outcomes in Children with Sickle Cell Disease. *N Engl J Med 2000;342:83-89.*

34 Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N Engl J Med 1994;330:1639-1644.*

35 Kinney TR, Sleeper LA, Wang WC, Zimmerman RA, Pegelow CH, Ohene-Frempong K, et al. Silent Cerebral Infarcts in Sickle Cell Anemia: *A Risk Factor Analysis. Pediatrics 1999;103:640-645.*

36 Castro O, Brambilla D, Thorington B, Reindorf C, Scott R, Gillette P, et al. The acute chest syndrome in sickle cell disease: incidence and risk factors. The Cooperative Study of Sickle Cell Disease. *Blood 1994;84:643-649.*

37 Lee SP, Ataga KI, Orringer EP, Phillips DR, Parise LV. Biologically Active CD40 Ligand Is Elevated in Sickle Cell Anemia. *Arterioscler Thromb Vasc Biol 2006;26:1626-1631.*

38 Henn V, Slupsky JR, Gräfe M, Anagnostopoulos I, Förster R, Müller-Berghaus G, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature 1998;391:591-594.*

39 Brambilla DJ, Miller ST, Adams RJ. Intra-individual variation in blood flow velocities in cerebral arteries of children with sickle cell disease. *Pediatric Blood & Cancer 2007;49:318-322.*

40 Brass LM, Pavlakis SG, DeVivo D, Piomelli S, Mohr JP. Transcranial Doppler measurements of the middle cerebral artery. *Effect of hematocrit. Stroke 1988;19:1466-1469.*

41 Adams RJ. Big Strokes in Small Persons. *Arch Neurol 2007;64:1567.*

42 Adams RJ, Nichols FT, McKie VC, McKie KM, Stephens S, Carl E, et al. Transcranial Doppler: Influence of hematocrit in children with sickle cell anemia without stroke. *J Cardiovascular Technology 1989;8:97–101.*

43 Lagunju I, Sodeinde O, Telfer P. Prevalence of transcranial Doppler abnormalities in Nigerian children with sickle cell disease. *Am J Hematol 2012;87:544-547.*

44 Melo HAd, Barreto-Filho JAS, Prado RCPd, Cipolotti R. Transcranial doppler in sickle cell anaemia: evaluation of brain blood flow parameters in children of Aracaju, Northeast - Brazil. *Arq Neuropsiquiatr 2008;66:360-364.*

45 Smits MAJ, van der Land V, Heijboer H, Fijnvandraat K. Blood flow velocities of intracranial arteries in children with sickle cell disease seem to decrease with age. *Tijdschr Kindergeneeskd 2013;81:109-109.*

46 Padayachee ST, Thomas N, Arnold AJ, Inusa B. Problems with implementing a standardised transcranial Doppler screening programme: impact of instrumentation variation on STOP classification. *Pediatr Radiol 2011;42:470-474.*

47 Adams RJ, McKie VC, Carl EM, Nichols FT, Perry R, Brock K, et al. Long-term stroke risk in children with sickle cell disease screened with transcranial doppler. *Ann Neurol 1997;42:699-704.*

48 Lagunju I, Sodeinde O, Brown B, Akinbami F, Adedokun B. Transcranial doppler ultrasonography in children with sickle cell anemia: Clinical and laboratory correlates for elevated blood flow velocities. *J Clinical Ultrasound 2013;42:89-95.*

49 Telfer P, Coen P, Chakravorty S, Wilkey O, Evans J, Newell H, et al. Clinical outcomes in children with sickle cell disease living in England: a neonatal cohort in East London. *Haematologica 2007;92:905-912.*

50 Galadanci NA, Abdullahi SU, Tabari MA, Abubakar S, Belonwu R, Salihu A, et al. Primary stroke prevention in Nigerian children with sickle cell disease (SPIN): Challenges of conducting a feasibility trial. *Pediatric Blood & Cancer 2014;62:395-401.*

51 Bernaudin F, Verlhac S, Arnaud C, Kamdem A, Chevret S, Hau I, et al. Impact of early transcranial Doppler screening and intensive therapy on cerebral vasculopathy outcome in a newborn sickle cell anemia cohort. *Blood 2011;117:1130-1140.*

52 Krejza J, Mariak Z, Walecki J, Szydlik P, Lewko J, Ustymowicz A. Transcranial color Doppler sonography of basal cerebral arteries in 182 healthy subjects: age and sex variability and normal reference values for blood flow parameters. *Am J Roentgenol 1999;172:213-218.*

53 Schöning M, Hartig B. The development of hemodynamics in the extracranial carotid and vertebral arteries. *Ultrasound Med Biol 1998;24:655-662.*

54 Seong J, Lieber BB, Wakhloo AK. Morphological age-dependent development of the human carotid bifurcation. *J Biomech 2005;38:453-465.*

55 Schöning M, Staab M, Walter J, Niemann G. Transcranial color duplex sonography in childhood and adolescence. Age dependence of flow velocities and waveform parameters. *Stroke 1993;24:1305-1309.*