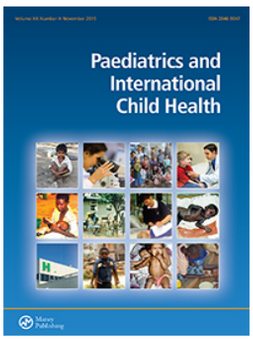

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Haemoglobin oxygen saturation, leucocyte count and lactate dehydrogenase are predictors of elevated cerebral blood flow velocity in Nigerian children with sickle cell anaemia

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ABSTRACT

Background: Transcranial Doppler ultrasound (TCD) scan, which measures blood flow velocity through the time-averaged mean of maximum velocities (TAMMVs) in the internal carotid arteries and middle cerebral arteries, is a useful screening tool for predicting stroke risk in children with sickle cell anaemia (SCA).

Aim: To investigate which clinical and laboratory indices predict abnormal TCD velocity in children with SCA.

Methods: Fifty-four SCA patients with normal TCD (TAMMV < 170 cm/s), classified as negative TCD (NTCD), and 93 patients with conditional and abnormal TCD velocities (TAMMV ≥ 170 cm/s) classified as positive TCD were recruited. The haemoglobin oxygen saturation, haematological variables, nitric oxide metabolites and lactate dehydrogenase activity of the patients were analysed.

Results: The mean (SD) age was 7.16 (3.84) years (range 2–16). The median SpO_2 of the patients in the positive TCD group was significantly lower than that of the negative TCD group ($p = 0.002$). Multivariate logistic regression analysis indicated that the MCV [odds ratio (OR) 1.12, 95% confidence interval (CI) 1.04–1.22, $p = 0.01$)], MCH (OR 1.34, 95% CI 1.02–1.77, $p = 0.04$), leucocyte count (OR 1.26, 95% CI 1.07–1.49, $p = 0.01$) and lactate dehydrogenase (LDH) level (OR 1.00, 95% CI 1.00–1.01, $p = 0.01$) were independent predictors of high cerebral blood flow velocities.

Conclusions: These clinical and laboratory indices are characteristic of chronic hypoxia and severe anaemia and are predictors of abnormal cerebral blood flow velocity. They can be used to predict stroke risk in children with SCA when access to TCD screening is limited.

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Transcranial Doppler; haemoglobin oxygen saturation; cerebral blood flow velocity; sickle cell anaemia; stroke risk; lactate dehydrogenase; laboratory predictors; Nigeria

Introduction

Stroke is an incapacitating neurological complication of sickle cell anaemia (SCA) that contributes significantly to childhood morbidity and mortality. In SCA, stroke results primarily from progressive narrowing of the medium and large blood vessels supplying the brain, especially the middle cerebral and distal internal carotid arteries. According to the Cooperative Study of Sickle Cell Disease, the incidence of cerebrovascular accident in the United States is 0.61/100 patient years compared with 1.3/100 patient years in Africa [1]. Eleven per cent of children with SCA will have a clinical stroke by the age of 20 years, with recurrence rates of 14.0–61.5% and the highest risk of stroke being between the ages of 2 and 9 years [1]. In Nigeria, a stroke prevalence of approximately 4–8% has been reported among children with sickle cell anaemia [2–5].

The increased cerebral blood flow that heralds stroke in children with SCA often progresses asymptomatically, but it can be detected by transcranial Doppler ultrasonography (TCD) before infarction. TCD measures the blood flow velocity in the large intracranial vessels of the circle of Willis. It has been validated as a useful screening tool for predicting the risk of stroke and measures blood flow velocity through time-averaged mean of maximum velocities (TAMMVs) in the internal carotid arteries and middle cerebral arteries [6]. Arterial stenotic lesions induce elevated blood flow velocity and are associated with an increased risk of stroke [7]. TCD scans can identify children with abnormal TCD velocity and target them for therapeutic intervention to prevent a primary stroke [8]. Two large, independent studies of children with SCA in the USA reported an approximately 10% prevalence of abnormal TCD [8,9], and a similar prevalence (9.3%) was reported in Nigeria [10].

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Studies in children with SCA have demonstrated that abnormal TCD findings (TAMMV ≥ 200 cm/s) are a predictor of stroke. Children with abnormal TCD scans have an approximately 44-fold greater risk of developing primary stroke than those with normal TCD velocities (TAMMV < 170 cm/s). Children with conditional TCD velocities (TAMMV of 170–199 cm/s) have a lower elevation of stroke risk than children with abnormal TCD velocities but they are still at significant risk [6,8]. The normal, conditional and abnormal stroke risk groups have been associated with a 2, 7 and 40% risk of stroke, respectively, within 3 years of undergoing an ultrasound screening test [11].

Despite the reliability of TCD as a stroke risk assessment tool, access in Nigeria has been limited by the cost of diagnosis and the availability of TCD ultrasonography. Nigeria is the country with the highest burden of SCA in the world [12]. An estimated 150,000 infants with SCA are born annually in Nigeria, and stroke represents an established cause of death [12]. There is, therefore, an urgent need for alternative cost-effective, safe and accessible diagnostic methods of predicting stroke in children with SCA. This study investigated the relationship between hypothesised clinical indices for stroke risk (haemoglobin oxygen saturation, haematological variables, nitric oxide metabolites and lactate dehydrogenase activity) and TCD velocities in children with SCA in Lagos.

Patients and methods

The study was conducted at the National Sickle Cell Centre in Lagos which provides routine clinical and laboratory services to SCA patients. It offers TCD screening services to children with SCA to identify those with a high risk of stroke so that primary stroke prevention with chronic blood transfusion and/or hydroxyurea therapy can be initiated. In a 3-month period, 461 patients attended the centre for TCD scan. The following were exclusion criteria: being on hydroxyurea therapy, patients with clinical stroke, patients who had received blood transfusion less than 4 months before the study, and patients who had vaso-occlusive crisis or fever. Of 418 patients eligible for the study, 249 had a standard risk, 114 conditional risk and 55 were at high risk. Fifty-four consecutive SCA patients with a standard risk, 51 with a conditional risk and 42 with abnormal risk were recruited. The age range of the study patients was 2–16 years.

All the patients were confirmed to be HbSS via haemoglobin high-performance liquid chromatography. HbSC patients were excluded from the study because the stroke risk stratification in the STOP trial procedure was not adequate to detect HbSC patients at risk of stroke [13].

Transcranial Doppler ultrasonography

TCD was performed according to the Stroke Prevention Trial in Sickle Cell Anaemia protocol [7] by a TCD technician who had received formal training in the use of the TCD machine and who performed the scan under the supervision of a consultant radiologist [10]. The patients were kept calm and were not allowed to sleep during the examination as carbon dioxide levels increase during sleep and elevate flow velocity, which could lead to a false positive result [14,15]. The angle of insonation (also known as Doppler angle is the angle between the ultrasound beam and the direction of blood flow) during the scan was kept between 0 and 30° [16]. The patients were enrolled and scanned between the hours of 8:00 and 11:00 am daily throughout the study period.

The blood flow velocities from the major cerebral arteries were measured through trans-temporal windows with a cylindrical transducer and a 2-MHz pulsed Doppler box (DB-0563, Compumedics, Germany) that uses non-imaging and non-invasive TCD techniques. Briefly, with the patient in a supine position and the head stabilised, a probe was held against the patient's temples and adjusted at varying depths to measure the highest recorded mean velocity in each artery. The time-averaged mean of maximum velocity (TAMMV, in centimetres per second) in 2-mm increments in the middle cerebral arteries (MCA), distal internal carotid arteries (ICA) and anterior cerebral arteries (ACA) were recorded from the optimised Doppler spectral waveforms. Children with a cerebral blood flow velocity <170 cm/s were considered to be at standard risk and were scheduled for repeat examination after 12 months; children with velocities between 170 and 199 and ≥200 cm/s underwent a repeat TCD examination after 1 week and were regarded as having conditional and abnormal TCD, respectively, if the repeat scan result was the same.

The conditional and abnormal categories were grouped into one stratum designated as 'positive TCD' (TAMMVs ≥ 170 cm/s) and 'negative TCD' (TAMMVs < 170 cm/s) [6,9].

Measurement of haemoglobin oxygen saturation

Haemoglobin oxygen saturation (SpO_2) was measured using a Nellcon non-invasive pulse oximeter (evo PulseOne, MD300C201, Beijing Technology, China).

Blood collection and laboratory analyses

Five millilitres (mL) of venous blood was collected from each patient immediately after the TCD scan and pulse oximetry measurements; three mL was collected in EDTA bottles for haematological analyses, and 2 mL was collected in heparin bottles for lactate dehydrogenase and nitric oxide metabolites assays. The blood samples were centrifuged immediately after collection, and the plasma obtained was stored in aliquots at –80 °C in an ultra-low freezer (Platinum 500, Angelantoni, Italy) before analysis. The full blood count was evaluated using an automated haematology analyser (Mindray, BC-2800). Haemoglobin fractions (HbF% and HbS%) were determined with

high-performance liquid chromatography using the β-thalassaemia short programme Variant II (Bio-Rad Laboratories, Hercules, CA, USA). The nitric oxide level was quantitated colorimetrically (SFRI Chemistry Analyser) at 540 nm by determination of its oxidised product, NOx (nitric oxide metabolite), using the QuantiChrom Nitric Oxide assay kit from BioAssay Systems (Hayward, CA, USA). NOx production was measured after reduction of nitrate to nitrite using the improved Griess method. Lactate dehydrogenase (LDH) activity was determined spectrophotometrically at 340 nm with a Cobas kit using a Roche/Hitachi analyser (Roche Diagnostics GmbH). Briefly, the rate of NADH decrease was measured, which is directly proportional to the presence of LDH which catalyses the inter-conversion of pyruvate and lactate.

Statistical analysis

The data are expressed as mean (SD) and median (interquartile range). The means of the negative and positive TCD groups were compared using the unpaired Student's *t*-test for normally distributed data and the non-parametric Mann–Whitney U-test for data which were not normally distributed. To further gain insight into the association between the TCD results and the examined variables, a multivariate logistic regression model analysis was performed. Data were analysed using GraphPad Prism 5.0 and the Statistical Package for Social Science (SPSS v.15) software; *p*-values < 0.05 were considered statistically significant.

Ethics

The study protocol was approved by the Health Research Ethics Committee of Lagos University Teaching Hospital (ADM/DCST/HREC/843). Informed assent and written informed consent were obtained from the patients and parents/guardians of each patient prior to enrolment.

Results

The study population was 147 SCA patients with a mean (SD) age of 7.16 (3.84) years. The TCD classification of these patients showed an association with age distribution ($\chi^2 = 12.36$, $p = 0.002$). In the positive and negative TCD groups, 53.8 and 24.1% of patients, respectively, were between 2 and 5 years.

The median SpO_2 of the patients in the positive TCD group was significantly lower than that of the patients in the negative TCD group ($p = 0.002$). In the patients in the positive TCD group, leucocyte count, MCV, MCH and LDH activity were significantly increased ($p < 0.05$), and haematocrit significantly reduced ($p < 0.05$) compared with those with negative TCD results (Table 1). Haemoglobin levels, platelet counts, nitric oxide metabolites and HbF were not significantly different between the two groups. Multivariate logistic regression analysis was undertaken to determine which variables are independent predictors of elevated TCD velocities. Of the eight variables included in the model, only four remained as independent predictors of high cerebral blood flow velocities: MCV (OR 1.12, 95% CI 1.04–1.22, $p = 0.01$), MCH (OR 1.34, 95% CI 1.02–1.77, $p = 0.04$), leucocyte count (OR 1.26, 95% CI 1.07–1.49, $p = 0.01$) and LDH (OR 1.00, 95% CI 1.00–1.01, $p = 0.01$) (Table 2).

Table 1. Clinical, haematological and biochemical data of the patients.

	Negative TCD	Positive TCD	
	n = 54	*n* = 93	*p*-value
SpO_2, %	98 [96–98]	97 [95–98]	**0.002**
Haemoglobin, g/dL	8.23 (1.07)	7.94 (0.83)	0.07
RBC, $\times 10^{12}$/L	3.22 (0.70)	2.95 (0.53)	**0.01**
HCT, %	26.16 (3.64)	24.84 (2.70)	**0.01**
MCV, fL	79.74 (7.44)	82.64 (7.73)	**0.03**
MCH, pg	25.54 (2.64)	26.56 (2.35)	**0.02**
MCHC, g/dL	31.84 (1.28)	32.27 (1.33)	0.06
Leucocytes, $\times 10^9$/L	13.09 (3.96)	16.82 (5.18)	**<0.0001**
Lymphocytes, %	43.14 (11.56)	43.43 (10.05), *n* = 92	0.87
Neutrophils, %	48.99 (11.53)	47.44 (12.27), *n* = 90	0.45
Platelets, $\times 10^9$/L	386.22 (141.22), *n* = 51	412.72 (123.37), *n* = 92	0.24
LDH, U/L	709.41 (249.51), *n* = 35	979.63 (404.06), *n* = 77	**<0.0001**
NOx, μM	32.82(14.96), *n* = 42	41.68 (27.99), *n* = 74	0.06
HbF, %	8.25 (5.13)	7.24 (4.48)	0.21
HbS, %	80.41 (6.80)	81.46 (8.39)	0.44

Note: Data are presented as mean (SD), except for SpO_2 [median (interquartile range)]. SpO_2, haemoglobin oxygen saturation; negative TCD, TAMMV < 170 cm/s; positive TCD, TAMMV > 170 cm/s; RBC, red blood cell counts; HCT, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; HbF, fetal haemoglobin; HbS, haemoglobin S; NOx, nitric oxide metabolite; LDH, lactate dehydrogenase. *p*-values in bold are statistically significant.

Table 2. Multivariate logistic regression of variables and TAMMV.

	β	SE	OR (95% CI)	*p*-value
Age	−0.12	0.098	0.89 (0.73–1.07)	0.21
SpO_2	−0.152	0.099	0.86 (0.71–1.04)	0.13
HCT	0.094	0.095	1.10 (0.91–1.32)	0.33
RBC	0.092	0.623	1.10 (0.32–3.72)	0.88
MCV	0.117	0.042	1.12 (1.04–1.22)	**0.01**
MCH	0.295	0.142	1.34 (1.02–1.77)	**0.04**
WBC	0.234	0.083	1.26 (1.07–1.49)	**0.01**
LDH	0.003	0.001	1.00 (1.00–1.01)	**0.01**

Note: *p*-values in bold are statistically significant.

Discussion

Sickle cell anaemia encourages the development of stroke by causing endothelial injury via the special effects of hypoxia, abnormal endothelial adherence of sickled red blood cells and inflammation [17,18]. It is well known that a number of factors are involved in the stenosis and infarction of the vasculature in sickle cell patients [19]. The endothelium regulates the pro- *vs* anti-coagulant balance of the blood vessels, regulates vascular pressure and flow, participates in inflammatory signalling pathways via adhesion molecules, and controls vessel wall

permeability [19]. When the endothelium is disrupted by these processes in SCA, vasculopathy increases the risk of cerebral infarction. In Nigeria, as in most other African countries, there is lack of a national newborn screening programme and as a result, SCA is usually diagnosed at the onset of sickle cell clinical presentations, such as hand-foot syndrome and other painful episodes, severe anaemia and stroke. In this study, 79.4% of the patients between the ages of 2 and 5 years were in the positive TCD group, and 53.8% of the patients in the positive TCD group were in this age group, indicating the high risk of stroke in younger patients despite the supposedly high levels of HbF in children under 5 years of age. This finding is supported by previous studies which reported that the highest incidence of first stroke was in younger age groups [1,20]. There is growing evidence that lower haemoglobin oxygen saturation can predict abnormal cerebral blood flow velocity (CBFv) and stroke risk [21]. CBFv, which refers to an increased volume of blood flow through the artery, can be caused by the reduction of the arterial diameter [22]. Haemoglobin desaturation decreases the arterial oxygen content, limiting oxygen delivery to the brain [23]. Multivariate analysis of Kenyan SCA patients which included age and haematocrit demonstrated that $SpO_2 \leq 95\%$ was associated with high cerebral blood flow velocity, similar to findings in this study [24]. This association of lower haemoglobin oxygen saturation with increased cerebral blood flow velocity suggests that taking account of SpO_2 measurements along with TCD velocity may improve the prediction of high risk of stroke in children with SCA. Because haematocrit is a major determinant of blood viscosity, the lower haematocrit observed in those in the positive TCD group may be explained by blood viscosity, which is decreased when the haematocrit level is reduced at a constant blood pressure and vessel lumen diameter, thus leading to elevated cerebral blood flow velocity [25].

High steady-state leucocyte count, a risk factor for stroke, results from the generation of an inert inflammatory response, which leads to the release of a cytokine mediator, thus increasing polymorphonuclear neutrophil production [17,26]. Leucocyte activation and adhesion to blood vessel walls are linked to endothelial dysfunction. Leucocytes interact with platelets and erythrocytes to form cell aggregates that subsequently obstruct the microvasculature, resulting in ischaemic organ damage and the development of SCA-related complications, such as stroke [27]. Haemoglobin desaturation in SCA alters endothelial function and activates leucocytes, red blood cells and adhesion molecules [28].

Decreased MCV and MCH have been associated with alpha-3.7 thalassaemia deletion; the association between alpha-3.7 thalassaemia deletion and a decreased risk of abnormal TCD and stroke in SCA patients could be the result of increased haemoglobin concentrations and reduced haemolysis associated with the co-inheritance of alpha-3.7 thalassaemia deletion [29]. The protective effect of alpha 3.7-thalassaemia deletion against cerebral vasculopathy has been reported in various studies [30–32]. The observed decrease in MCV and MCH in patients with negative TCD compared with those in the positive TCD group may suggest the influence of decreased MCV/MCH as surrogate of alpha-3.7 thalassaemia deletion in SCA patients [33].

Nitric oxide (NO) is an important regulator of vascular function and is produced by the endothelium. Assessments of NO metabolites are critical to understanding various physiological processes [34]. In SCA, a reduced NO level contributes to vasculopathy, erythrocyte adhesion, platelet activation and aggregation, and the activation of endothelial adhesion molecules [35]. NO is an unstable compound and is therefore determined by measuring nitric oxide metabolites (NOx) including nitrate and nitrite levels. The presence of higher NOx levels in patients with positive TCD findings compared with the negative TCD group demonstrates the increased depletion of NO. During intravascular haemolysis, erythrocytes are lysed and release cell-free haemoglobin to consume NO; this limits NO metabolites, leading to vasculopathy and the narrowing of large intracerebral vessels and may account for the near-significant increase in NOx in the patients in the positive TCD group compared with those in the negative TCD group [36]. It should be noted, however, that NOx measurement is difficult and there are many confounders such as dietary intake and artefactual formation or nitrite contamination in laboratory wares [37].

In patients with SCA, LDH is considered to be a significant biomarker of intravascular haemolysis. Previous studies of SCA have shown that high LDH is associated with vaso-occlusive crises, pulmonary hypertension, leg ulceration and priapism [34,38]. In this study, a significantly higher LDH level was observed in the patients with positive TCD findings than in those with negative TCD findings and further established the correlation between increased TCD velocity and higher LDH activity. This finding corroborates the results of previous findings that indicated a correlation between TAMMV and LDH and suggests that intravascular haemolysis may be the basis of high cerebral blood flow velocity in SCA patients [13,39].

Although chronic blood transfusion has been shown to reduce the risk of stroke, few patients in Nigeria with abnormal TCD undergo chronic blood transfusion. The major impediments to successful chronic blood transfusion therapy, particularly in sub-Saharan Africa, are the unavailability of blood for regular transfusion, poor access to comprehensive treatment centres, the cost of transfusion and the socio-economic burden on families. Additionally, the risk of allo-immunisation, iron overload and transfusion-transmissible infections cannot be over-emphasised. Consequently, in Nigeria, hydroxyurea therapy is more commonly used for primary stroke prevention than chronic transfusion.

This study could not follow the participants to identify high-risk patients who may develop stroke despite hydroxyurea therapy and/or chronic blood transfusion. The clinical and laboratory predictors found in this study are limited to TCD because the study did not include data on SCA patients with stroke.

In conclusion, lower haemoglobin oxygen saturation, higher leucocyte count and lactate dehydrogenase activity in steady-state patients can predict increased cerebral blood flow velocity in children with SCA. These predictors of elevated TCD velocity are suggestive of chronic hypoxia and severe anaemia, which are risk factors for stroke in patients with SCA. They can be used to predict stroke risk in children with SCA in sickle cell clinics when access to TCD screening is limited.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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