Factors Associated with Elevated Transcranial Doppler Ultrasound Velocities in Children With Sickle Cell Anemia in Mwanza, Tanzania

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Abstract

Background: Stroke occurs in 11% of patients with SCA before 20 years of age. In Northwestern Tanzania, the prevalence of stroke among children living with SCA under the age 15 years is 16.9%, of which might be attributed to the absence of routine screening for the risk of stroke by using Transcranial Doppler Ultrasound (TCD). Screening with TCD allows preventive measures such as chronic blood transfusion to be done which has led to the reduction of stroke by 92%.

Methods: This was a prospective analytical cross sectional study which enrolled 267 SCA children aged 2 to 16 years attending Bugando Medical Centre Pediatric Sickle Cell Clinic from July 2019 to June 2020. Assessment of factors associated with elevated TCD included a clinical history of stroke in sibling, death in sibling, temperature, oxygen saturation in room air, blood pressure, hemoglobin level and total white blood cell count. TCD was done by accessing transtemporal window and recording the highest time average mean of maximum velocity (TAMMV) of major vessels mainly, middle cerebral artery (MCA) and distal internal carotid artery (dICA).

Results: The median age of enrolled was 6.6 (IQR: 4-9) years. The prevalence of elevated TCD (> 170 cm/s) was found to be 21% (56/267). By multivariate logistic regression, low oxygen saturation in room air, \( p \)-value = 0.037, OR 1.08 [95% CI 1.00-1.17] and low hemoglobin level, \( p \)-value = 0.001, OR 1.76 [95% CI 1.26-2.45] were statistically significantly associated with elevated TCD among children living with SCA.

Conclusion: The high prevalence of elevated TCD velocity, with low hemoglobin and low oxygen saturation in room air as associated factors under multivariate logistic regression, warrants routine TCD screening for children with SCA aged 2 to 16 years.

Introduction

Sickle Cell Anemia (SCA) is a disorder constituting a substitution of an amino acid, glutamine with valine, after a single base pair change of thymine for adenine at the 6th codon of the \( \beta \) globin gene in chromosome 11 [1]. This substitution causes the red blood cell to change into a sickle shape, specifically when a child/patient is under hypoxic conditions because of polymerization of the hemoglobin molecules [1]. As one of the major complications, cerebral vascular diseases may manifest into an overt. The development of stroke is linked to the deformity of the red blood cells whereby the lysed red blood cells release arginase and other adhesions molecules that lead to reduced level of nitrous oxide [2,3] this vicious cycle results in poor flow and finally stroke.

Tanzania ranks fourth worldwide among countries affected by sickle cell disease [4]. The high disease burden is mostly seen in the Lake Zone regions of the country [5,6]. Despite being highly affected with SCA and having a prevalence of stroke of 16.9% in SCA (carrying HbSS) children less than fifteen years of age [7], with BMC being a zonal consultant hospital in the Lake Zone offering sickle cell clinic services, still, screening by using TCD is not routinely being practiced. This creates a gap in offering standard stroke prevention care which in turn will not only reduce mortality and morbidity but also reduce cost to the health system and the child’s family.


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Prevalence of abnormal TCD screening has been fluctuating in several studies. Meanwhile, in the STOP trial reported a prevalence of abnormal TCD of 6.72% [8] that of a study done in Kenya showed 0% had abnormal TCD with conditional TCD being 3% [9], eliciting the need to fully understand the situation in our setting.

The study specific objectives were to determine the prevalence of elevated TCD velocities and factors associated with elevated TCD velocities in children with SCA.

Methods

Study area

This study was done at Bugando Medical Centre (BMC) Outpatient Sickle Cell Clinic, a zonal consultant and teaching hospital with 950 bed capacity. It caters to the Lake Zone (with a geographic area of 11,4348 km²) which has a population of 16 million from the following regions; Kagera (25,265 km²), Geita (20,054 km²), Mwanza (9,467 km²), Shinyanga (18,901 km²), Simiyu (18,901 km²) and Mara (21,760 km²) [10,11]. This study was done at Bugando Medical Centre (BMC).

Study population

The study population included children with sickle cell anemia aged 2 to 16 years attending BMC pediatric outpatient sickle cell clinic. Age range was selected based on report that sickle cell stroke affects children and adolescents aged 2 to 20 years [13].

Eligibility criteria

The inclusion criteria was children aged 2 to 16 years and homozygous SS. Exclusion criteria was children who had received blood less than 2 weeks before the date of enrollment, febrile illness in the past 2 weeks, inpatient, patients who were in hydroxyurea therapy and patients with stroke (as previously described; this poses a challenge especially in young children. If a child is restless, sleeping or agitated not only makes it difficulty accessing the transtemporal windows but also can cause elevation of the TAMMV of MCA or dICA) were placed in a comfortable position, during examination were placed in a comfortable position, laying supine on a bed allowing them to watch television playing soothing kids' shows or cartoons while the guardian or parent was present during the examination. Inadequate examinations were documented but excluded from the study. The average screening time with TCD was thirty to forty minutes. The TCD examination was supervised and validated by a qualified radiologist at BMC conversant in TCD ultrasonography.

Informed consent was obtained to partake in the study, then evaluation for the inclusion and exclusion criteria was done. For those enrolled, a clinical examination and laboratory checklist form was used to collect information (demographic data, temperature, oxygen saturation, blood pressure, hemoglobin level, total white blood cell count and TCD screening findings).

A structural clinical and laboratory standardized data collection tool was used to collect demographic, clinical, capture laboratory data and maximum TCD velocity for each participant. Demographic data collected entailed age, gender, history of stroke in sibling and death history in a sibling. Clinical data included; temperature, oxygen saturation by a pulse oximeter machine and blood pressure. Then a physical examination was performed followed by a Transcranial Doppler ultrasonography, which recorded the TAMMV (Time Averaged Mean of Maximum Velocity) of the bilateral middle cerebral and internal carotid arteries.

Assessment of elevated TCD velocities

The TCD examination was performed using a non-imaging TCD ultrasonography machine-VIASYS Healthcare, serial label SN: PVK0254 with a Doppler Box with DWL Doppler system and QL software using a 2MHZ single element handheld non-imaging pulsed transducer, this was placed at the temporal regions (trans temporal) of the child. Measurements of the major arteries of the circle of Willis (MCA and dICA) were read from both sides of the head (left and right) following the STOP protocols for stroke prevention trial in SCA (STOP) [14]. The TCD machine records a non-imaging spectrum whereby the x axis captures the time with the y axis reporting on the velocity as centimeters per second (cm/s). The highest recorded velocity was obtained from TAMMV as a final reading.

TCD has been recommended as tool for assessing risk of first stroke [15]. Even though TCD has shown to have a low predictive value of 40% with a sensitivity of 59% and specificity of 95% [16] similar efficacy rates were reported by Hoppe, et al. [17], it has led to a significantly drop of in the prevalence of stroke in children with SCA after detection of risk of stroke and chronic blood transfusion was initiated [18]. It requires the subject to remain awake and calm during the procedure as previously described; this poses a challenge especially in young children. If a child is restless, sleeping or agitated not only makes it difficulty accessing the transtemporal windows but also can cause elevation of the TAMMV of MCA or dICA [18,19].

In order to reduce this operational challenge, children during examination were placed in a comfortable position, laid supine on a bed allowing them to watch television playing soothing kids' shows or cartoons while the guardian or parent was present during the examination. Inadequate examinations were documented but excluded from the study. The average screening time with TCD was thirty to forty minutes. The TCD examination was supervised and validated by a qualified radiologist at BMC conversant in TCD ultrasonography.

The TCD examination was done with no cost to the participant. Children with elevated findings were enrolled to
ancillary study for further follow up for a period of 3 years including TCD, enrollment to hydroxyurea treatment and laboratory parameters.

**Laboratory methods**

Blood sample was collected by veno puncture (phlebotomy) and a 4mls sample collected in a purple top EDTA collecting tube. The tube was correctly labeled by the participant ID code, date taken, and name of the collector and it was clearly marked it is for purpose of this study by the letters TCD. Then it was stored in a cold box at room temperature and transported to BMC laboratory after enrollment with results of hemoglobin and total white blood cell count recorded.

**Data management and statistical analysis**

Data was entered into Microsoft excel database, analyzed by using STATA version 13, prevalence of elevated TCD velocity among SCA children was determined, with factors associated with elevated TCD velocities in SCA children determined by univariate logistic regression followed by multivariate logistic regression for factors with $p$ value < 0.2 on univariate regression with odd ratio, 95% confidence interval and $p$ value < 0.05 and was considered statistically significant.

**Ethical considerations**

Ethical clearance for the research proposal was granted by the joint BMC/CUHAS ethics and review committee with research clearance number CREC/389/2019. Furthermore, written informed consent was ensued to the parents/guardians of children with SCA attending at BMC clinic after they have clearly understood the objective of the study by using an information sheet and consent form. Assent was sought for children above the age of 7 years, the parents or guardians signed the informed consent form. For illiterate parents/guardians, a fingerprint was used instead of a signature.

**Results**

**Study enrollment**

The study was conducted from July 2019 to June 2020. Within the study period a total of 1021 SCD patient visits were attended in the Pediatric Sickle cell clinic. After screening for eligibility, 267 SCA children aged 2 to 16 years were enrolled into the study (Figure 1).

**Baseline characteristics**

The median age was 6.6 (interquartile range (IQR): 4 - 9) years with a mean ± SD (96.6 ± 3.5 years). Females were 137 (51.3%). Only one participant had a sibling with sickle cell stroke as reported by the parent (0.4%). (Table 1).

**Outcomes**

The prevalence of elevated TCD was 21% (56/267). Among those who were screened 211 (79.0%) had normal TCD (≤170 cm/s), 52 (19.5%) had conditional TCD (171 – 200 cm/s) and only 4 (1.5%) had abnormal TCD (≥200) (Figure 2). The overall TAMMV mean ± SD (145.5 ± 25.8 cm/s) and for elevated TCD velocity mean ± SD (181.9 ± 11.2 cm/s).

In univariate logistic regression, factors associated with elevated TAMMV of TCD ultrasound included high body

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**Table 1: Baseline participants’ characteristics.**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Total of children screened = 267</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, Mean ± SD</td>
<td>6.62 ± 3.45</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>137 (51.3)</td>
</tr>
<tr>
<td>Sibling with stroke</td>
<td></td>
</tr>
<tr>
<td>Yes (%)</td>
<td>1(0.4)</td>
</tr>
<tr>
<td>No (%)</td>
<td>266(99.6)</td>
</tr>
<tr>
<td>Death history in sibling</td>
<td></td>
</tr>
<tr>
<td>Yes (%)</td>
<td>34(13)</td>
</tr>
<tr>
<td>No (%)</td>
<td>233(87)</td>
</tr>
<tr>
<td>Temperature(°C), Mean ± SD</td>
<td>36.48 ± 0.51</td>
</tr>
<tr>
<td>Oxygen saturation-SPO2 (%), Mean ± SD</td>
<td>93.58 ± 3.90</td>
</tr>
<tr>
<td>Systolic blood pressure -SBP (mmHg), Mean ± SD</td>
<td>98.38 ± 9.56</td>
</tr>
<tr>
<td>Diastolic blood pressure-DBP (mmHg), Mean ± SD</td>
<td>60.44 ± 7.26</td>
</tr>
<tr>
<td>Hemoglobin (g/dl), Mean ± SD</td>
<td>7.90 ± 1.34</td>
</tr>
<tr>
<td>Total white blood cell count [x109/L], Mean ± SD</td>
<td>13.81 ± 5.61</td>
</tr>
<tr>
<td>Median age in years for elevated TCD (interquartile range)</td>
<td>6 (4-9.5)</td>
</tr>
<tr>
<td>Median age in years for abnormal TCD (interquartile range)</td>
<td>6.5 (5 - 7)</td>
</tr>
</tbody>
</table>
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When compared to other countries apart from the sub-Saharan Africa, a higher prevalence of elevated TCD was observed. In Brazil, Leite, et al. and Silva, et al. reported the prevalence of elevated TCD of 11% [25] and 14% [26] respectively. These lower elevated TCD velocity were also observed at baseline by Bernaudin, et al. in France whereby 10% had elevated TCD [27].

The findings seen in this study for elevated TCD velocity are higher than those seen in the Americas and in France. However, with a conversion rate of 34.5% and a median time of conversion of about 1.1 (range 0.03 - 7) years from conditional to abnormal TCD in SCA as reported by Bernaudin, et al. [27], it can be speculated that there is a higher cumulative risk of conversion to abnormal in our setting and therefore a higher risk of stroke, although a longitudinal cohort study is required to verify this claim.

Factors associated with elevated TCD in SCA

This study showed on univariate regression high temperature, low hemoglobin level, high total white blood cell count and low oxygen saturation in room air were significantly associated with elevated TCD, with multivariate regression eliciting only low hemoglobin level and low oxygen saturation in room air to be associated with elevated TCD.

Several studies have reported analogous findings to this study. In a study done in Muhimbili National Hospital Sickle Cell Clinic located in Tanzania Central Zone whereby low hemoglobin correlated with high TCD [28]. In addition, in Kenya a positive relation to high TCD was observed between oxygen saturation ≤95%, fever and TCD of ≥150 cm/s [9]. Meanwhile Nigeria showed increased cerebral flow velocities with low hemoglobin, low hematocrit and arterial oxygen desaturation [24]. Another study reported that overt stroke is associated with low daytime oxygen desaturation levels [29].

In Brazil, low levels of hemoglobin, high leucocyte count (with no infection) were associated with high baseline TCD [26]. Also in France, abnormal TCD was less in patients with high hemoglobin and more in those with high white blood cell count [20]. These findings are similar to those reported in this study.

Table 2: Factors associated with elevated TCD in SCA children aged 2 to 16 years.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Elevated N = 56</th>
<th>Normal N = 211</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
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<tbody>
<tr>
<td>Age, years (IQR)</td>
<td>6 (4 - 9.5)</td>
<td>6 (4 - 9)</td>
<td>1.00 [0.92 - 1.09]</td>
<td>0.937</td>
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<tr>
<td>Temperature, °C ± SD</td>
<td>36.5 ± 0.6</td>
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<td>SBP*, mmHg ± SD</td>
<td>99.6 ± 10.6</td>
<td>98.1 ± 9.3</td>
<td>1.02 [0.99 - 1.05]</td>
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<td>DBP*, mmHg ± SD</td>
<td>60.0 ± 7.7</td>
<td>60.6 ± 7.15</td>
<td>0.99 [0.95 - 1.03]</td>
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<td>SPO2* ,% (IQR)</td>
<td>93 (90 - 95)</td>
<td>94 (92 - 97)</td>
<td>0.87 [0.82 - 0.95]</td>
<td>0.001 0.92 [0.85 - 1.0]</td>
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<td>Hemoglobin, g/dl</td>
<td>7.2 ± 1.1</td>
<td>8.1 ± 1.3</td>
<td>0.51 [0.38 - 0.69]</td>
<td>&lt;0.001 0.57 [0.41 - 0.79]</td>
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<td>Total white blood cell count, x 10^9/L (IQR)</td>
<td>15.6 (12.9 - 17.3)</td>
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<td>Gender</td>
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<td>30(54%)</td>
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<td>Death history in sibling</td>
<td>9(16%)</td>
<td>25(12%)</td>
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Bolded results are statistically significant at p ≤ 0.05 by Multivariate logistic regression, OR denoted Odds ratio and 95% CI denote 95% confidence interval.

Discussion

Prevalence of elevated TCD

Sickle cell anemia carries a risk of stroke with eleven percent of children and adolescent acquiring stroke before the age of twenty years [20]. The elevated TCD prevalence in this study is higher than that reported in a study done in Kenya, whereby only 3% had elevated TCD velocity [9]. The difference might be attributed to usage of different TCD machines and mortality differences. Children in Kenya have a high mortality rate and therefore die at a very young age thus failed to be captured [21]. This can be further explained as in the Kenya study none of the children were received penicillin prophylaxis or hydroxyurea [9] insinuating premature death due to sepsis or SCA complications while penicillin prophylaxis is offered to under five SCA children at BMC pediatric sickle cell clinic, with hydroxyurea therapy reserved for those with severe complications.

Similar findings have been observed from studies in Mali and Nigeria with the prevalence of elevated TCD 18% [22] and 24% [23] respectively. A subsequent study in Nigeria showed even higher prevalence of 30% [24]. This reflects the burden of the disease in Sub Saharan Africa, with Tanzania’s Lake Zone showing a higher prevalence of sickle cell trait and subsequently HbSS [5] in our country. In addition, the diversity of genetic β cluster haplotypes distribution [21] among the African countries might also have role in predisposition to elevated TCD and the fluctuations in the prevalence among different countries, although this was not assessed and future studies are needed to evaluate genetic predisposition in our setting.

When compared to other studies the prevalence of elevated TCD was higher in this study. In a study done in Muhimbili National Hospital Sickle Cell Clinic located in Tanzania Central Zone whereby low hemoglobin correlated with high TCD [28]. In addition, in Kenya a positive relation to high TCD was observed between oxygen saturation ≤95%, fever and TCD of ≥150 cm/s [9]. This study showed on univariate regression high temperature, low hemoglobin level, high total white blood cell count and low oxygen saturation in room air were significantly associated with elevated TCD, with multivariate regression eliciting only low hemoglobin level and low oxygen saturation in room air to be associated with elevated TCD.

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Furthermore, elaborating on the linkage between low hemoglobin and elevated TCD in SCA, low hemoglobin level, as a marker of ongoing hemolysis can be an indication of potential vascular injury. The pathological injury occurs as a cascade of events which start or precipitated by hemolysis with a release of red blood cell components into the system including free heme, iron, and reactive oxygen species, vasoactive peptides with platelet and white blood cell aggregation and adhesion to the endothelial wall [30-32].

As a result, the bone marrow responds by production of reticulocytes, which have a higher propensity to adhesion to vascular endothelial wall and thus the pathological endothelial injury viscous cycle is propagated [32] until flow is obstructed and stroke ensue. This pathophysiological injury has also been reported to occur in nocturnal deoxygenation [33]. Additionally, oxygen saturation has been reported to be inversely associated with TCD velocity [34] which further support that the deoxygenation state of hemoglobin contributes to the pathophysiological vessel injury in the brain.

Familial predisposition to stroke has been reported in several studies. In this study only 0.4% reported on familial history of stroke as history of a sibling with stroke. This low prevalence was also seen in Brazil whereby it was 1% [25]. Also due to only one history of sibling with stroke, the statistical correlation of history of stroke in sibling and elevated TCD was not ascertained.

Limitations

TCD was done by trained and certified clinicians then validity assessed by an experienced radiologist, this might subject the study to measurement bias of which was minimized by improving accuracy by following the STOP protocol. The absence of neuroimaging studies failed to report on cerebral vasculopathy in elevated TCD and inadequate windows.

Additionally, TCD screening on average took around thirty to forty minutes secondary to ensuring thorough STOP protocol is followed, the vessels velocity is optimized and the participant is calm and not sleeping. This made time restriction a factor for enrolling participants.

Conclusion

The high prevalence of elevated TCD velocity, with low hemoglobin and low oxygen saturation in room air as associated factors warrants implementation of routine TCD screening for children with SCA aged 2 to 16 years.

Recommendations

Routine TCD velocity screening for children aged 2 to 16 years of children living with SCA. In addition, hemoglobin level and oxygen saturation at room air should be incorporated into the screening tools in addition to TCD and also checked in routine clinic visits with rescreening TCD done if abnormal levels seen in order to ameliorate risk of stroke. Lastly, future longitudinal cohort with randomized sampling should be done in our setting for capturing the cause and effect of associated factors of elevated TCD.

Disclaimer

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