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# Studies on Transcranial Doppler Ultrasound Velocities and their Association with Hemoglobin F Expression in Saudi Arabian Pediatric Sickle Cell Patients

Oncology

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## ABSTRACT

Sickle cell disease (SCD) is an inherited disease which leads to production abnormal hemoglobin. Clinical presentation may include vaso-occlusive, hematological and infectious crises. One of the common complications in SCD is stroke. Prevention of the first stroke (primary prevention) is possible by assessing the risk of stroke by transcranial Doppler (TCD) ultrasound and blood transfusions to patients with elevated TCD velocities. No studies were previously carried out in Saudi Arabia about higher TCD velocities in SCD. Further, although higher HbF expression is also associated with less severity of the SCD, no studies are available about association of HbF with TCD flow velocities are hence risk of stroke in SCD. This study has been carried out to identify SCD patients at high risk of stroke based on TCD results, and to determine the association between Hb-F levels and blood velocity using TCD results. A cross-sectional study carried out on 56 pediatric SCD, TCD ultrasounds were carried out initially at diagnosis to assess the risk for stroke and repeated after every 6 months for follow-up studies. Patients with TCD velocities higher than 199 cm/sec were enrolled in monthly packed RBCs transfusions to have HbS level around 40%. Data analysis was performed by "Statistical Package for the Social Sciences (SPSS, Version 17.0)". Results revealed that in In our patient population, male to female ratio was 1.33:1 (32 males, 24 females) and mean age was 7.5 ± 3.42 years (range: 2-14 years). Seven (12.5%) patients had TCD velocities higher than or equal to 200 cm/second, with mean age of 4.7±1.4 years and mean HbF 7.5±3.8, which were significantly different from patient group with normal flow velocities (mean age of 7.8±3.4 years; mean HbF 20±15.9). Patients with higher TCD velocities receiving blood transfusion got velocities in normal range within 6-12 months. Most importantly, normal TCD flow velocities were significantly associated with higher HbF expression and vice versa. In conclusions our results showed low prevalence of SCD patients with higher TCD velocities which were clinically manageable by 6-12 months of regular PRBCs transfusion to prevent strokes. Moreover, normal TCD flow velocities and hence lower risk of stroke was significantly associated with the higher hemoglobin F expression and vice versa.

## KEYWORDS:

## INTRODUCTION

Sickle cell disease (SCD) is an inherited red blood cell disorder that leads to presence of abnormal hemoglobin with limited oxygen carrying capacity [1]. The clinical presentations may include vaso-occlusive, hematological and infectious crises [2]. SCD has a worldwide distribution. In the United States, 72 000 people are suffering from SCD and 2 million are carriers while median age of death is 53 years for men and 58 years for women [3, 4]. However, SCD patients in US are still hospitalized frequently [3, 4]. Overall incidence of SCD in Saudi Arabia is 4.2% with highest frequencies in eastern province [5]. SCD frequencies in different regions of Saudi Arabia are: Qatif (eastern region) 17.0 %, Gizan (southern region), 10.3%, Ula (Northern region) 8.1 % and Mecca (western region) 2.5 % [2, 5].

One of the common complications of SCD is stroke which is one of the major causes of morbidity and mortality in patients with SCD [6]. Prevention of strokes is possible in at risk patients by timely assessment of the risk of stroke by transcranial Doppler (TCD) ultrasound and preventive treatment by blood transfusions [7, 8]. Prognostic stratification of SCD by using TCD ultrasound for assessing the risk of stroke and subsequent differential treatment has led to very significant reduction in the strokes and other morbidities associated with SCD (9). TCD results are classified as normal, conditional, abnormal or inadequate based on velocity readings in specific arterial segments [8, 9]. Flow velocities of 200 cm/sec in the middle cerebral or the internal carotid arteries has been reported to be associated with 40% risk of developing a stroke while flow velocities of <170 cm/sec and 170–199 cm/sec are associated with a stroke-risk of 2 and 7%, respectively [8-10]. There are no published reports about TCD flow velocities and their association with risk of stroke in Sickle cell anemia from Saudi Arabia .

Furthermore, some of the recent reports indicate higher expression of fetal hemoglobin F (HbF) in the sickle cell patients with normal TCD flow velocities [11, 12]. It is well known that HbF expression is associated with milder symptoms and improved long-term survival in SCD patients [13]. There are no reports if HbF is associated with lower normal TCD flow velocities. Therefore, aim of this study was to identify proportion of pediatric SCD patients at King Abdulaziz Medical City, Riyadh who are at high risk of stroke based on TCD results, and to determine the association between Hb-F levels and blood velocity using TCD results.

## MATERIALS AND METHODS

This study was conducted at "Department of Hematology/Oncology" and "Department of Medical imaging", King Abdul-Aziz Medical City (KAMC), Riyadh, Saudi Arabia. Pediatric Sickle cell anemia patients diagnosed with homozygous sickle cell anemia attending department of hematology/oncology from January 2010 to January 2013 were included in the study. This inclusion criterion is based on "International Classification of Disease (ICD-9-CM)" [14]. Initially, TCD ultrasound was carried out at diagnosis for risk stratification [15, 17]. TCD results were classified on the basis of time-averaged mean of the maximum velocity (TAMMvel) in the cerebral artery and interpreted as follows: "normal," all mean velocities less than 170 cm/sec; "conditional," at least 1 mean velocity of 170 to 199 cm/sec with none greater than or equal to 200 cm/sec; "abnormal," at least 1 mean velocity of 200 cm/sec or higher [8, 9 &15].

Patients were divided into two groups on the basis of TCD ultrasound results: SCD patients with normal TCD flow velocities (Group 1) and SCD patients with high TCD flow velocities (Group 2). Patients with TCD velocities higher than 199 cm/sec were enrolled in monthly packed RBCs transfusions to have HbS level around 40% [15, 17]. TCD ultrasounds were repeated after every 6 months for follow-up studies [15, 17]. Data analysis was performed by "Statistical Package

for the Social Sciences (SPSS, Version 17.0)". The number and percentage was calculated for categorical and continuous variables. The categorical variables include sex, and TCD results. The continuous variables were age and HbF. Chi-square was the test of choice for categorical variable. P-value < 0.05 was concluded to be statically significant. Patient consent was not needed as this was retrospective chart review study, approved by King Abdullah International Medical Research center. Confidentiality of the patients was observed.

## RESULTS

The study population consisted of 56 patients had diagnosed with Homozygous sickle cell anemia. Mean age of the patients was 7.5 ± 3.42 years (range: 2-14 years). There were 32 males and 24 females (1.33:1 ratio). The mean HbF of the patients was 19.96 ± 15.91.

### SCD patients with normal TCD flow velocities (Group 1):

Forty nine patients (87.5%) had TCD flow velocities less than 170 cm/second (Table 1). Patients in this group had mean age of 7.8±3.4 years and mean HbF 20±15.9. Their mean TCD flow velocities were well below 170 cm/second. None of patients in this group experienced any strokes.

### SCD patients with high TCD flow velocities (Group 2):

Out of 56, 7 (12.5%) patients were found to have TCD velocities higher than or equal to 200 cm/second (Table 2). None of the patients had TCD velocities from 170-199 cm/second. Mean age of the patients in this group was 4.7±1.4 years and mean HbF was 7.5±3.8, which were significantly different from group 1 patients (Table 1). Follow up using TCD ultrasound after 6 months showed blood velocity less than 170 cm/second in 4 patients while remaining 3 patients' TCD velocities became less than 170 cm/sec, after one year of regular PRBCs transfusion. This indicates that TCD can detect risk of first stroke efficiently in SCD patients and those monthly packed RBCs transfusions can clinically manage risk of stroke in SCD patients.

Our results showed low prevalence (12.5%) of higher TCD velocities in SCD patients and risk of stroke could be prevented by 6-12 months of regular PRBCs transfusions. Moreover, normal TCD flow velocities and hence lower risk of stroke was significantly associated with the higher hemoglobin F expression and vice versa.

**Table 1:** Comparison of mean age, Hemoglobin-F levels (%) and TCD flow velocities of the major intracranial arteries (cm/sec) in sickle cell patients with normal TCD flow velocities (Group 1) and sickle cell patients with high TCD flow velocities (Group 2).

| | N | Age | HbF | RMCA | LMCA | RACA | LACA | RPCA | LPCA |
|---|---|---|---|---|---|---|---|---|---|
| Group 1 | 49 | 7.8 ±3.4 | 20 ±15.9 | 108.9 ±23.2 | 112.5 ±25.1 | 77.9 ±22.2 | 83.9 ±22.3 | 67.04 ±20.4 | 70.38 ±21.4 |
| Group 2 | 7 | 4.7 ±1.4 | 7.5 ±3.8 | 175.7 ±27.6 | 193.7 ±34.9 | 94.7 ±26.2 | 93.3 ±23.4 | 84.3 ±18.4 | 84.3 ±26.5 |
| p values | - | 0.02 | 0.04 | 0.0001 | 0.0001 | 0.07 | 0.3042 | 0.03 | 0.12 |

TCD: trans cranial Doppler; N: Number of patients; HbF: Hemoglobin F; RMCA: Right middle cerebral artery; LMCA: Left middle cerebral artery; RACA: Right anterior cerebral artery; LACA: Left anterior cerebral artery; RPCA: Right posterior cerebral artery; LPCA: posterior cerebral artery.

**Table 2:** Hemoglobin-F levels (%) and TCD flow velocities of the major intracranial arteries (cm/sec) in 7 SCD patients with elevated TCD flow velocities (Group 2)

| Patients Number | Gender | Age (years) | HbF | RMCA | LMCA | RACA | LACA | RPCA | LPCA |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 5 | 5% | 200 | 226 | 78 | 140 | 60 | 130 |
| 2 | M | 7 | 15% | 150 | 200 | 90 | 75 | 75 | 100 |
| 3 | F | 4 | 6% | 170 | 220 | 100 | 85 | 110 | 95 |
| 4 | M | 3 | 10% | 180 | 200 | 85 | 95 | 100 | 80 |
| 5 | M | 4 | 7% | 200 | 150 | 150 | 68 | 90 | 50 |
| 6 | F | 6 | 4.3% | 200 | 140 | 70 | 90 | 65 | 65 |
| 7 | F | 4 | 5% | 130 | 220 | 90 | 100 | 90 | 70 |
| Mean | - | 4.7 ±1.4 | 7.5 ±3.8 | 175.7 ±27.6 | 193.7 ±34.9 | 94.7 ±26.2 | 93.3 ±23.4 | 84.3 ±18.4 | 84.3 ±26.5 |

TCD: transcranial Doppler; N: Number of patients; HbF: Hemoglobin F; RMCA: Right middle cerebral artery; LMCA: Left middle cerebral artery; RACA: Right anterior cerebral artery; LACA: Left anterior cerebral artery; RPCA: Right posterior cerebral artery; LPCA: posterior cerebral artery.

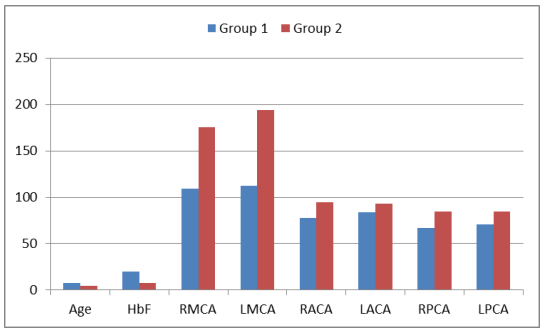


Fig. 1: Comparison of mean age, Hemoglobin F levels (%) and TCD flow velocities of the major intracranial arteries (cm/sec) in sickle cell patients with normal TCD flow velocities (Group 1) and sickle cell patients with high TCD flow velocities (Group 2).

TCD: Trans cranial Doppler; N: Number of patients; HbF: Hemoglobin F; RMCA: Right middle cerebral artery; LMCA: Left middle cerebral artery; RACA: Right anterior cerebral artery; LACA: Left anterior cerebral artery; RPCA: Right posterior cerebral artery; LPCA: posterior cerebral artery.

## DISCUSSION

Though Saudi Arabia has one of the highest incidence of sickle cell disease in the world [2, 5] and many of these patients are at high risk of first stroke as indicated by higher TCD flow velocities clinically manageable by blood transfusions [6-9], no studies have previously been carried out in Saudi Arabia generally and at King Abdul-Aziz Medical City (KAMC) Riyadh specifically about frequency of higher transcranial Doppler (TCD) flow velocities and management of at stroke risk group in sickle cell patients. We here reported the frequency of TCD flow velocities in SCD patients, their association with hemoglobin F and outcome of transfusion-based management of SCD patients at risk of first stroke.

Our results showed 7 (12.5%) of SCD patients with higher TCD velocities. Adams *et al*. [15] reported higher TCD velocities in SCD patients to be 12.1%, which supports our study. They also reported patients with higher TCD velocities to be younger than patients with normal TCD flow velocities (7.3±3.2 versus 9.1±4.2), which is in accordance with our results (Table 1). However, mean age of our patients with higher TCD velocities is significantly lower than that reported by Adams *et al*. (4.7±1.4 versus 7.3±3.2; p-value=0.047). Nevertheless, very recently Bernaudin *et al.* [9] have found much higher frequencies of higher blood flow velocities in SCD patients (29.8%), which indicate that frequencies of TCD flow velocities can differ in different ethnic groups. However, mean age of patients with higher velocities in their study is similar to our group of SCD patients (3.7 ±1.5 versus 4.7±1.4; p-value=0.09).

TCD velocities became less than 170 cm/sec after 6-12 months in our patients who received transfusions and none of these patients got stroke during follow up of 6 years, which is supported by studies from Bernaudin *et al.* [9] in which no stroke occurred in sickle cell patients with elevated velocities who received blood transfusions during a median Follow up of 6.1 years. Nevertheless, in STOP-1 trial, one patient receiving transfusion got stroke [18, 19]. This proves that blood transfusion is a very valuable strategy to minimize stroke risk in sickle cell patients with abnormal TCD flow velocities.

In our studies, normal TCD flow velocities and hence lower risk of stroke was significantly associated with the higher hemoglobin F expression while higher TCD flow velocities were significantly associated with lower hemoglobin F expression. Asbeutah *et al* .[12] reported that high HbF expression in SCD patients with less severe disease and not presenting early although a positive correlation between high TCD flow velocities and lower HbF expression was not found. As protective role of HbF in sickle cell patients is well established [11,13] and inducing the HbF expression in such patients

using medications like hydroxyurea [20] or by genetically modifying the silencing of fetal γ-globin gene have been long standing goal in treatment of sickle cell disease [21], our findings about correlation of higher TCD flow velocities with lower HbF expression (and vice versa) and hence higher risk of first stroke in sickle cell patients provides a new direction for assessing the risk for vasculopathy in SCD patients. Nevertheless, as due to our smaller population size, we recommend our findings to be confirmed in studies involving large patient populations.

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