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Study of transcranial Doppler ultrasound and endothelin-1 in children with sickle cell anemia: a single-center study

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To assess the impact of our transcranial Doppler (TCD) screening program on the incidence of a first stroke in children with sickle cell anemia and to study the role of elevated serum endothelin-1 (an inflammatory mediator) in these children. Background: stroke is a major complication of sickle cell disease (SCD), even in very young children. About 11% of children with homozygous sickle cell anemia (SS) develop stroke by the end of the second decade of life. The underlying etiology in most cases is an ischemic stroke caused by large-vessel stenosis or occlusion. Transcranial Doppler (TCD) recommended as a routine screening test to identify children at high risk of developing a stroke, measures flow velocities within large intracranial arteries. TCD should be routinely performed in children between 2 and 16 years as this age group is at the highest risk of sickle cerebral vasculopathy. We carried out a prospective case-control study which included 2 groups: a patient group consisted of 30 children with sickle cell anemia and sickle thalassemia and a group of 30 healthy children of matched age and sex. Each group included 11 males (36.5%) and 19 females (63.5%); the age range was 2 to 17 years. Both groups underwent a thorough clinical examination and laboratory tests (CBC, liver and renal function, serum ferritin and endothelin-1). Additionally, TCD was performed in all children included in the patient group. According to the results of TCD, time-averaged mean of the maximum velocity (TAMMX) was < 170 cm/s (normal), $170\text{--}200$ cm/s (conditional), ≥ 200 cm/s (high risk) in 20 (66.7%), 4 (13.3%) 6 (20%) patients, respectively. The level of endothelin-1 was significantly higher in the patients (57.1 ± 91.3) than in the controls (21.9 ± 14.8). Hemoglobin concentration was significantly lower in the patient group than in the control group, but the levels of reticulocytes, WBCs and serum ferritin were significantly higher in the patients than in the healthy controls. Serum Endothelin-1 level was higher in patients with sickle cell anemia than control group.

Key words: *transcranial Doppler, endothelin-1, children and sickle cell anemia*

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Sickle cell disease is an inherited RBC disorder primarily affecting people of African descent, and characterized by hemolytic anemia, vaso-occlusive events, and vasculopathy leading to multiorgan damage and early mortality. Sickle cell disease is caused by a mutant form of hemoglobin known as sickle hemoglobin (HbS) that arises from replacement of glutamic acid with valine in position 6 on β -globin subunit of hemoglobin [1].

Sickle cell anemia (SCA; homozygous sickle haemoglobin (HbS), i.e. HbSS) occurs when thymine is substituted for adenine in the 6th codon of the beta globin gene, resulting in the production of valine (a hydrophobic amino acid) instead of glutamic acid, which is hydrophilic. Although all SCA patients share the same genetic mutation, the clinical course is highly variable between patients [2].

The Transcranial Doppler examination is performed using a 2 MHz frequency ultrasound probe. The higher frequency probes used in extracranial Doppler studies are not applicable for intracranial measurements

because higher frequency waves are not able to adequately penetrate through the skull. In addition to using a lower frequency probe, insonation of the cerebral arteries is only possible through thinner regions of the skull, termed acoustic windows. Therefore, familiarity with the anatomic location of cerebral arteries relative to the acoustic windows and blood flow velocities for the various arteries is critical for accurate blood flow measurements through the nonduplex mode [3].

Neurologic manifestations are common in SCD and include overt infarction or stroke, silent cerebral infarcts, and hemorrhage (rare in children, more often seen secondary to aneurysm rupture in adults). Stroke results in neurologic deficits, and silent cerebral infarcts can result in academic and behavioral difficulties and increase the risk of having a future stroke [4].

Patients present with vasculopathy involving the large intracranial arteries, most frequently the distal internal carotid artery and proximal middle and anterior cerebral arteries. Vasculopathy can progress

for months or years before symptoms develop and can result in the formation of numerous small collateral vessels, consistent with moyamoya syndrome [5].

Indicators of transcranial Doppler in children with sickle cell disease:

- TAMM velocity, $MCA \geq 170$ cm/sec;
- Maximum velocity in PCA, vertebral, or basilar arteries greater than or equal to MCA velocity;
- Turbulence;
- Visualization of PCA or ACA without visualization of MCA;
- Any $RI < 0.3$;
- Maximum MCA PSV ≥ 200 cm/sec [6].

Endothelin-1, a potent long-acting vasoconstrictor, counteracts the effects of nitric oxide in response to inflammatory stimuli, hypoxia, and shear stress. ET-1 is induced by sickle red blood cells *in vitro* and mediates reactive oxygen species generation through RBC NADPH oxidase activity. Treatment with an ET-1 receptor antagonist lowered RBC-associated protein disulfide isomerase oxidant activity in sickle mice [7].

Levels of ET-1 are increased in sickle cell disease patients during acute vaso-occlusive pain episodes and may remain elevated for weeks thereafter, suggesting that it may be partly responsible for prolongation of vaso-occlusive pain symptoms [8].

MATERIALS AND METHODS

A prospective case-control study was carried out at Menoufia University Hospital and El-Helal Hospital over a period of two years from December 2016 to April 2018.

The study was approved by the local ethics committee of Menoufia University and written consent was obtained from the parents.

All children's mothers gave their consent to the following:

- Complete history;
- Clinical examination;
- Laboratory investigations:
 1. Routine investigations:
 - complete blood picture;
 - kidney function test;
 - liver function tests;
 - serum ferritin
 2. Specific investigation (TCD – Serum Endothelin1).

Statistical analysis

The data collected were tabulated and analyzed by SPSS (Statistical Package for the Social Sciences) Version 20 on IBM compatible computer.

The results were presented by applying ranges, mean \pm SD, X-square test, Mann-Whitney test, Kruskal-Wallis test and *p*-values. The *p*-value ≤ 0.05 was considered to be significant.

Pearson correlation was used for normally distributed quantitative variables, while Spearman correlation was used for quantitative variables that were not normally distributed or when one of the variables was qualitative.

RESULTS

The study included a total of 60 male and female participants: sickle cell anemia patients and apparently healthy children. In the group of patients, there were 11 males (36.5%) and 19 females (63.5%). Fourteen patients (46.7%) had a positive history of consanguinity, 16 (53.3%) patients had a negative history of consanguinity. Mean \pm SD values of weight and height in the patient group were 18.83 ± 5.5 kg and 116.43 ± 20.15 cm, respectively. In the control group, 5 children (16.7%) had a positive consanguinity, 25 children (83.3%) had a negative consanguinity; mean \pm SD values of weight and height were 22.63 ± 7.55 kg and 117.7 ± 19.73 cm, respectively (table 1).

Based on transcranial Doppler ultrasound, we divided the patients into three groups according to time-averaged mean of the maximum velocity (TAMMX): high risk TCD (6 patients (20%)), conditional TCD (4 patients (13.3%)), normal TCD (20 patients (66.7%)) (table 2).

In the patients, the laboratory findings were as follows: hemoglobin concentration (g/dl): 9.7 ± 1.7 ; WBC count ($\times 10^3/\text{mm}^3$): 8.9 ± 2.9 ; platelet count ($\times 10^3/\text{mm}^3$): 394.6 ± 90.9 ; reticulocyte count (%): 3.61 ± 1.13 ; serum ferritin level (ng/ml): 368.5 ± 802.9 . In the controls, the laboratory findings were as follows: hemoglobin concentration (g/dl): 11.5 ± 1.4 ; WBC count ($\times 10^3/\text{mm}^3$): 6.7 ± 1.9 ; platelet count ($\times 10^3/\text{mm}^3$): 253.3 ± 113.5 ; reticulocyte count (%): 0.58 ± 0.25 ; serum ferritin level (ng/ml): 44.4 ± 16.3 .

Thus, hemoglobin concentration was significantly lower in the patient group than in the control group. The level of reticulocytes, WBCs, creatinine, serum ferritin and SGOT were significantly higher in the patients than in the healthy controls, but there was no significant difference regarding MCV, MCH, platelets and SGPT (table 3).

The rate of blood transfusions was 100%, 50%, and 25% in the high-risk group, conditional group, and normal group, respectively). Therefore, the number of blood transfusions was significantly higher in the patients from the high-risk TCD group than in the patients from the conditional and normal TCD groups, but there was no significant difference regarding splenectomy or drug history (table 4).

Endothelin-1 concentration was significantly higher in the patient group (57.1 ± 91.3 ng/dl) than in the control group (21.9 ± 14.8 ng/dl) (table 5).

Table 1
General characteristics of the study group

	Case (n = 30)		Control (n = 30)		Test of sig.	p-value
	n	%	n	%		
Gender						
Male	11	36.5	11	36.5	X ² = ----	p = ----
Female	19	63.5	19	63.5		
Age						
Mean ± SD	7.7 ± 3.5		6.7 ± 3.4		Mann–Whitney = 1.3	p = 0.21 (> 0.05)
Range	2–15		2–15			
Residence						
Rural	17	56.7	19	63.5	X ² = 0.28	p = 0.79 (> 0.05)
Urban	13	43.3	11	36.5		
Consanguinity						
Yes	14	46.7	5	16.7	X ² = 6.2	p = 0.00 (HS) (≤ 0.001)
No	16	53.3	25	83.3		
Weight (kg)						
Mean ± SD	18.83±5.5		22.63 ± 7.55		t = 2.227	p = 0.030 (< 0.05) (S)
Range	10–30		12–39			
Height (cm)						
Mean ± SD	116.43 ± 20.15		117.7 ± 19.73		t = 0.246	p = 0.806 (> 0.05)
Range	80–155		86–160			

Note. NS – non-significant; S – significant.

Table 2
Transcranial Doppler ultrasound in sickle cell anemia patients

Item	Frequency (n = 30)	Percentage, %
TCD		
High-risk (more than 200 cm H ₂ O)	6	20
Conditional (170–199 cm H ₂ O)	4	13.3
Normal (170 or less cm H ₂ O)	20	66.7

Note. TCD – transcranial Doppler; Based on the results of transcranial Doppler ultrasound, the patients were divided into three groups: high-risk TCD (6 patients, 20%), conditional TCD (4 patients, 13.3%) and normal TCD (20 patients, 66.7%).

DISCUSSION

Sickle cell disease (SCD) is the most frequent cause of stroke in black children and the pathophysiology of stroke in SCD is complex [9].

Children who had significant vessel narrowing on cerebral angiography demonstrated an abnormally high TCD blood flow velocity (≥ 200 cm/s) in their cerebral arteries, particularly in the middle cerebral arteries (MCA) and/or in the internal carotid arteries (ICA) [10].

In our study, the weight of sickle cell anemia patients was significantly lower than that of the controls (table 1).

This result is in agreement with the findings reported by Rees et al. (2008) who showed that clinical and hematological characteristics such as weight and hemoglobin level in Nigerian children with sickle cell anemia were lower than in controls [11].

The patients' school performance was significantly lower than that of the controls (table 1).

These results are consistent with the results of King et al. (2014) who reported that patients with

sickle cell anemia, especially those with high time-averaged mean of the maximum velocity (TAMMX) on TCD, demonstrated decreased school performance and IQ-test scores. When other factors that contribute to school performance were examined as part of the Silent Cerebral Infarct Multicenter Clinical Trial (SIT trial), they observed decline in patients than controls.

Based on the results of transcranial Doppler ultrasound, the patients were divided into three groups: high-risk TCD (6 patients, 20%), conditional TCD (4 patients, 13.3%) and normal TCD (20 patients, 66.7%) (table 2).

These results are in agreement with those obtained by Arkuszewski et al. (2012) who reported about 110 patients in their cohort study with a total of 291 TCD examinations [12]. These patients had a mean age of 7.6 years with a range from 2–18 years of age. Of the 291 TCD examined, 46 (16%) patients had high risk TCD by TAMV criteria. One hundred and sixteen (40%) patients had conditional TCD, and 129 (44%) patients had normal transcranial doppler.

In the current study, hemoglobin levels were significantly lower in the patients than in the healthy controls; on the contrary, reticulocyte and WBC counts were significantly higher in the patients than in the control group. However, there was no significant difference regarding MCV, MCH and platelets between the two groups (table 3).

These results agree with the results of Abdullahi et al. (2014) who showed that the main hematological findings in their study of children with sickle cell anemia were moderate anemia, leukocytosis, reticulocytosis and increased platelet counts (at the

Table 3

A comparison of laboratory testing results of the patients and the controls

Items	Patients (n = 30)	Controls (n = 30)	Test of sig.	p-value
Hb (g/dl)				
Mean ± SD Range	9.7 ± 1.7 7–13	11.5 ± 1.4 9–14	Mann–Whitney = 3.9	p = 0.001 (S)
MCV (fl)				
Mean ± SD Range	82.3 ± 4.7 71–89	82.93 ± 6.2 71–90	t = 1.006	0.319 (NS)
MCH (pg)				
Mean ± SD Range	28.50 ± 2.67 23–39	27.97 ± 2.45 23–31	t = 2.016	0.058 (NS)
Reticulocytes (%)				
Mean ± SD Range	3.61 ± 1.13 1–5	0.58 ± 0.25 0.50–1	t = 14.352	p = 0.001 (S)
WBCs × 10 ⁹ /l				
Mean ± SD Range	8.9 ± 2.9 13–13.5	6.7 ± 1.9 3.5–9.9	t = 3.4	p = 0.001 (S)
PLT (× 10 ⁹ /l)				
Mean ± SD Range	394.6 ± 90.9 128–518	253.3 ± 113.5 190–454.7	Mann–Whitney = 1.3	p = 0.21 (NS)
Urea (mg/dl)				
Mean ± SD Range	20.8 ± 6.8 9–37	21.2 ± 3.8 16–29	Mann–Whitney = 0.54	p = 0.56 (NS)
Creatinine (mg/dl)				
Mean ± SD Range	0.70 ± 0.47 0–1	0.93 ± 0.25 0–1	Mann–Whitney = 2.3	p = 0.021 (S)
SGPT (U/l)				
Mean ± SD Range	30.6 ± 15.9 15–102	24.5 ± 6.4 13–36	Mann–Whitney = 1.6	p = 0.12 (NS)
SGOT (U/l)				
Mean ± SD Range	31.2 ± 17.3 13–81	23.2 ± 7.04 13–42	Mann–Whitney = 2.03	p = 0.04 (S)
Serum ferritin (ng/ml)				
Mean ± SD Range	368.5 ± 802.9 23–4318	44.4 ± 16.3 15–77	Mann–Whitney = 4.5	p = 0.001 (S)

Note. SGOT – serum glutamate oxaloacetic transaminase; SGPT – serum glutamic pyruvic transaminase; Hb – hemoglobin; WBC – white blood cell; PLT – platelets; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; t – student t-test; S – significant; NS – non-significant.

Table 4

A comparison between different patient categories regarding blood transfusion, surgical and drug history

Items	High-risk TCD (n = 6)		Conditional TCD (n = 4)		Normal TCD (n = 20)		Test of sig.	p-value
	n	%	n	%	n	%		
Blood transfusion								
Yes	6	100	2	50	5	25	X ² = 10.6	p = 0.005 (S)
No	0	0	2	50	15	75		
Surgical history (splenectomy)								
Yes	0	0	0	0	1	5	X ² = 0.52	p = 0.77 (NS)
No	6	100	4	100	19	95		
Hydroxyurea								
Yes	5	83.3	3	75	14	70	X ² = 0.43	p = 0.81 (NS)
No	1	16.7	1	25	6	30		
Hydroxyurea duration in months								
Mean ± SD	61 ± 32.3		57 ± 40.8		34 ± 30.7		Kruskal–Wallis = 4.4 p = 0.110(NS)	
Range	36–120		24–108		1–108			
L-carnitine								
Yes	6	100	4	100	20	100		
No	0	0	0	0	0	0		

Note. X² – Chi square; NS – non-significant; S – significant (p ≤ 0.05).

upper limit of the normal range) compared to the controls [13].

The number of blood transfusions was significantly higher in the patients from the high-risk TCD group than in the patients from the conditional and normal

TCD groups, but there was no significant difference regarding splenectomy or drug history (table 4).

These results are consistent with those of Pegelow et al. (2001) who reported an increased rate of blood transfusions in a high-risk group, as regular blood

Table 5
A comparison of endothelin-1 levels in the patients and the controls

Items	Patients (n = 30)	Controls (n = 30)	Test of sig.	p-value
Endothelin-1 (ng/dl)				
Mean \pm SD Range	57.1 \pm 91.3 8–432	21.9 \pm 14.8 8–86	Mann–Whitney = 2.5	p = 0.013 (S)
	High-risk (n = 6)	Conditional (n = 4)	Normal TCD (n = 20)	Test of sig./p-value
Endothelin-1 (ng/dl)				
Mean \pm SD Range	36.7 \pm 17.4 12–55	46.3 \pm 41.6 11–100	65.4 \pm 110.2 8–86	Kruskal–Wallis = 0.332 p = 0.89 (NS)

Note. S – significant; NS – non-significant.

transfusions in high-risk patients with sickle cell anemia decreased the risk of stroke.

Endothelin-1 level was significantly higher in the patients than in the controls, but there was no significant difference between different patient categories (table 5).

These results agree with the findings obtained by Prado et al. (2013) who reported increased endothelin-1 levels in children with sickle cell anemia, as they studied the role of endothelin-1 as an

inflammatory mediator released in response to vaso-occlusive crises in sickle cell anemia due to vascular injury.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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