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ORIGINAL ARTICLE

Endothelial Dysfunction Linked to Ventricular Dysfunction in Children With Sickle Cell Disease, a 3D Speckle Tracking Study

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Abstract

Background: **Sickle Cell Disease (SCD) is not a hematologic disease that occurs in isolation; it results in multi-organ complications. There is growing evidence of vascular stiffness as its underlying cause. This study aimed to investigate the relationship between endothelial stiffness and LV dysfunction in SCD patients and to explore its pathophysiology, particularly regarding the depletion of vasodilators such as Nitric Oxide (NO).**

Methodology: **32 patients with established criteria for SCD and 40 healthy control subjects were selected for this case-control study. Comprehensive clinical assessment and assessment of endothelial function using Brachial Flow-mediated dilation (FMD) were performed, along with serum NO measurement, which was followed by diagnosis and echocardiographic assessment using 3D speckle tracking echocardiography (STE) and tissue Doppler imaging (TDI).**

Results: **Collected SCD cases showed echocardiographic features of Systo-diastolic dysfunction with reduced FMD compared to controls, denoting endothelial dysfunction in those patients. LDH showed a marked elevation, while serum NO showed a significant reduction in cases compared with controls. We also noted a positive correlation between FMD on the one hand and measures of ventricular dysfunction and level of serum NO on the other hand, the latter proving that reduction of NO is responsible for reduced endothelial function.**

Conclusion: **We present the first report to date to outline the role of vascular stiffness as measured by brachial FMD in the induction of left ventricular dysfunction in SCD. We recommend that more research be conducted regarding possible strategies to replenish serum NO stores to delay microvascular injury and, in turn, ventricular dysfunction in SCD.**

Keywords: SCD, Microvascular dysfunction, Nitric oxide, Flow-mediated dilation, Speckle tracking, Myocardial injury

1. Background

Sickle cell disease (SCD) is a hereditary, life-threatening disease most commonly found in the pediatric age group, where the red blood cells have a reduced life span and are rigid, with a crescent or sickle shape, causing episodes of vaso-occlusion and hemolysis, which are ultimately responsible for the clinical manifestations of the disease [1].

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Cardiovascular complications are considered the leading cause of mortality in SCD patients. Unlike beta-thalassemia major, there is less need for frequent blood transfusions and lower iron load in SCD [2]. There is an increasing body of evidence that the leading cause of myocardial injury in SCD is related to vascular stiffness involving the coronaries. Such vascular stiffness is related to a decrease in circulating vasodilators, notably Nitric Oxide (NO) [3].

While Right Ventricular (RV) dysfunction in SCD is understandable because of the pressure overload caused by pulmonary vasculopathy, Left Ventricular function remains debatable in the context of SCD. Several studies have reported normal vs. hyperkinetic LV functions in SCD patients [4], whereas other studies have shown impaired functions [5,6]. This dysfunction mainly involves the subendocardial portion of the myocardium, as shown in our previous report using the transmural strain technique.

We postulate that the vascular stiffness observed in SCD patients might play a role in inducing LV dysfunction by initiating microvascular coronary involvement with subsequent myocardial ischemia.

Thus, the primary outcome parameter of this study was to confirm or exclude the presence of endothelial dysfunction in SCD patients, whereas the secondary outcome parameter was to relate such dysfunction to circulating NO levels and myocardial functions in these patients.

Abbreviations

ASD	Atrial septal defect
E/E'	Left Ventricular ratio of early diastolic mitral inflow velocity to the average of early diastolic velocities of the mitral annulus and basal septum
FMD	Flow-mediated dilation
GLS	Global longitudinal strain
LDH	Lactate dehydrogenase
NO	Nitric oxide
PFO	Patent foramen ovale
SCD	Sickle cell disease

2. Patients and methods

2.1. Study subjects

This case-control study was conducted at the Hematology Outpatient Clinic of Cairo University Children's Hospital (CUCH). It includes two groups:

Group 1: 32 patients with an established diagnosis of SCD who attended regular follow-up visits at the hematology clinic at Cairo University Children's Hospital. Exclusion criteria included the presence of any known structural heart disease apart from PFO/ASD. No sample size was performed at baseline, as the initial aim was to include every SCD patient following in our service and due to the scarcity of cut-off values of serum NO levels between SCD cases and controls. Alternatively, post hoc power analysis was planned after the results were available to calculate the statistical power of the study.

Group 2: 41 age- and sex-matched healthy controls who did not have any chronic illnesses. They were selected from the outpatient clinic of Cairo University Children's Hospital after the assessment and exclusion of significant acute or chronic illnesses. Controls were retrieved from well-child (regular check-up clinics), and the reason behind the fact that the controls' number exceeds the number of cases is to increase the matching of the two study groups.

Informed consent from all patients' guardians was obtained before data collection and sample withdrawal.

The study protocol was approved by the Pediatrics Department, Faculty of Medicine, Cairo University; it was held in July 2018.

2.2. Study methods

2.2.1. Clinical assessment

All included patients were subjected to Ref. [1] comprehensive history-taking and [2] complete clinical examination (general and systemic examinations), with special attention to anthropometric measurements such as body weight (kg), height (cm), and heart rate.

Our working group collected data on SCD complications suggestive of endothelial dysfunction following the methodology recommended by Van Der Land et al. and applied them to the Youssry et al. series.

Manifestations of endothelial dysfunction were expressed as follows:

- Frequency of vaso-occlusion events per patient per year (in the past 3 years).
- Any new pulmonary infiltrate associated with symptoms was considered an episode of acute chest syndrome.
- Leg ulcers manifested in affected subjects at the time of examination [7,8].

2.2.2. Assessment of endothelial functions using Brachial Flow-mediated dilation (FMD)

Measurement of FMD was performed according to the standard guidelines for assessment of the brachial artery. A longitudinal axis of the brachial artery was obtained while the patient was

maintained in the supine position for 5 minutes or more. Supra-systolic compression (50 mmHg above systolic blood pressure) was applied for another 5 minutes, and the artery diameter was measured 30 seconds before and 2 minutes after the release of pressure. The diameter was estimated as the distance between the anterior media-adventitia and the posterior intima-media [5].

2.2.3. Serum NO measurement immediately at diagnosis

Blood sampling and initial centrifugation were performed on the day of blood collection at the Central Laboratory of the Cairo University Children's Hospital.

Serum NOx concentration was measured using the Griess reaction, which is considered the most accurate method for the measurement of serum NO.

Zinc sulfate was used to deproteinize the sample (15 mg/mL). 100 μL of the supernatant was placed in a microplate well where 100 μL vanadium (III) chloride (Aldrich®) (8 mg/mL) was used for reduction of nitrate to nitrite. Griess reagents composed of 50 μL sulfanilamide (2 %) and 50 μL N-(1-Naphthyl) ethylene diamine dihydrochloride (Sigma®). (0.1 %) were mixed with the modified samples followed by an incubation for 30 min at 37 °C. A validated enzyme-linked immunosorbent assay (ELISA) reader (Sunrise, Tecan, Austria) was then used to measure absorbance at 540 nm. Serum NOx concentration was determined as a deviation from the linear standard curve at a scale from 0 to 100 μM sodium [9].

2.2.4. Echocardiographic assessment

Echocardiography was performed using General Electric (Vivid-7/N95, Horten, Norway). The latter allows 3D assessment of the left ventricle volumes; it also possesses a Tissue Doppler module. Examination was conducted according to the guidelines of the American Society of Cardiology as follows [10]:

- Conventional and Tissue Doppler Echocardiography for calculation of E/E′ (Left Ventricular ratio of early diastolic mitral inflow velocity to the average of early diastolic velocities of the mitral annulus and basal septum) as a potential measure of LV diastolic function.
- Three-Dimensional (3D) Echocardiography: LV volumes were obtained by full-volume mode in the apical view. Two points (at the apex and the base was placed to allow the software to track the LV motion in diastole and systole. This tracking allowed the software to deduct the LV volumes, Ejection Fraction, and 3D Global Longitudinal Strain.

3. Statistical methods

All collected data were analyzed using Medcalc statistical software. Continuous numerical variables are presented as mean and standard deviation (SD), and intergroup differences are compared using the independent-sample *t*-test. Categorical data were presented as numbers and percentages. Correlations were illustrated as scatter plots, as an expression of univariate regression analysis, and provided a correlation coefficient as well as a *P*-value.

Power analysis was done on all compared outcome variables. Student's t-test for independent samples was chosen to perform the power analysis, the α-error level was fixed at 0.05 and the sample size was entered to be 73 participants divided into 2 groups with controls to cases ratio = 1.29.

4. Results

Table 1 outlines the demographic and clinical characteristics of the two study groups. All collected cases were matched with controls for age 12.3 ± 3.7 and the mean of control was 12.2 ± 2.7. Males were the predominant gender in both study groups, accounting for 53% of cases and 56% of controls.

The clinical characteristics of the patients (Table 2) were consistent with microvascular dysfunction in the form of leg ulcers and a history of vaso-occlusive episodes. Nine patients had leg ulcers, and two-thirds of the patients had more than one episode of acute chest syndrome per year.

Table 1. Demographic data of cases and controls.

Variable		SCD (n = 32)	Controls (n = 41)	*P*-value
		Mean ± SD	Mean ± SD	
Age (years)		12.3 ± 2.7	12.2 ± 2.7	0.87
Sex (N/%)	Male	17 (53)	23 (56)	0.7
	Female	15 (47)	18 (44)	
Weight (kg)		34.3 ± 4.4	35.0 ± 4.2	0.3
BSA (m^2)		1.15 ± 0.1	1.17 ± 0.09	0.3
HR (beats per min)		92 ± 4	90 ± 3	0.6

Abbreviations: BSA: Body surface area; HR: heart rate.

Table 2. Hematologic characteristics of study cases.

Frequency of blood transfusion (mL/kg/year)	145 ± 16
Vaso-occlusive events per year	5 ± 2
Leg ulcers (number of patients)	9
Acute chest syndrome (number of patients with>1 episode in the preceding year)	20

Abbreviations: BSA (Body Surface Area), kg (Kilogram), mL (Milliliters).

Table 3 shows a comparison of the measures of ventricular function and brachial FMD between cases and controls. The cases showed echocardiographic features of systolic–diastolic dysfunction compared to the controls.

LV E/E', a measure of LV diastolic dysfunction, was significantly higher in cases than in controls (9 ± 2 vs. 4 ± 1). LV 3D EF and LV GLS were markedly reduced in cases compared with controls, indicating LV systolic dysfunction in the studied patients.

FMD was reduced in cases compared to controls reflecting endothelial dysfunction in SCD patients.

Table 4 demonstrates the discrepancy in the levels of biochemical markers between cases and controls. LDH, a marker of intravascular hemolysis, showed a marked elevation in cases compared to controls (760 ± 46 vs. 123 ± 16), and serum NO levels were significantly reduced in cases compared to controls (50 ± 15 vs. 215 ± 61).

Figs 1–3 are three scatter plots showing a statistically significant correlation between FMD on one hand and all the measures of ventricular dysfunction, namely LV EF, LV GLS, and LV E/E' ratio on the other hand.

Fig. 4 shows a statistically significant positive correlation between the serum NO levels and brachial FMD. The latter finding signifies that the reduction in NO is responsible for reduced endothelial function.

Statistical power for all compared outcome variables (except demographic characteristics and heart

Table 3. 3D Echocardiographic data, tissue Doppler data and flow mediated dilation data of the two study groups.

	SCD (n = 32)	Controls (n = 41)	P value
	Mean ± SD	Mean ± SD	
LV EDVI	67 ± 5	55 ± 4	<0.001
LV GLS (%)	17.1 ± 2.4	24.4 ± 4	<0.0001
EF	58.4 ± 5.7	65 ± 5.6	
LV E/E'	9.1 ± 2.1	4.8 ± 1.4	<0.0001
FMD	11.5 ± 2.7	19.9 ± 3.6	<0.0001

Abbreviations: EDVI (End-diastolic volume index), EF (Ejection Fraction), FMD (Flow mediated dilation), GLS (Global Longitudinal Strain), LV E/E' (Ratio of early transmitral flow velocity to average early diastolic velocities of the mitral annulus and basal septum), LV (Left Ventricle), SD (Standard deviation).

Table 4. Biochemical data of the two study groups.

	SCD (n = 32)	Control (n = 41)	*P*-value
	mean ± SD	mean ± SD	
LDH (U/L)	760 ± 46	123 ± 16	<0.0001
NO level (μmol/L)	50.3 ± 15.0	215 ± 61	<0.0001

Abbreviations: LDH (Lactate Dehydrogenase), L (Liter), mL (milliliter), ng (nanogram), NO (Nitric Oxide), SCD (Sickle Cell Disease), μmol (micromole). U (unit).

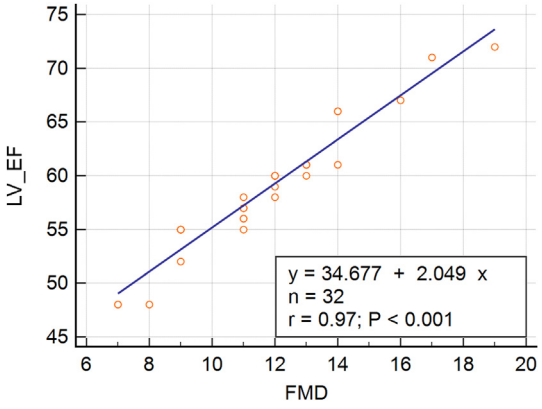

Fig. 1. Scatter plot for illustration of the correlation between LV EF and FMD. Abbreviations: EF: Ejection Fraction, FMD: Flow-mediated dilation, LV: Left Ventricle.

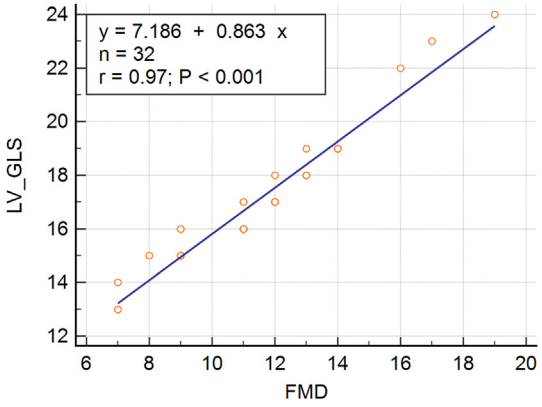

Fig. 2. Scatter plot for illustration of the correlation between LV GLS and FMD. Abbreviations: FMD: Flow-mediated dilation, GLS: Global longitudinal strain, LV: Left Ventricle.

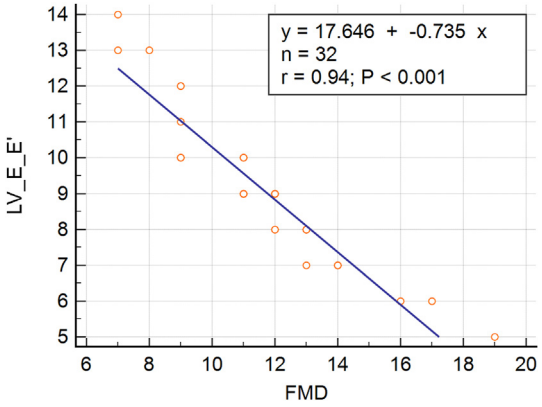

Fig. 3. Scatter plot for illustration of the correlation between LV E/E' and FMD. Abbreviations: FMD: Flow-mediated dilation, LVE/E': Left Ventricular ratio of early diastolic mitral inflow velocity to the average of early diastolic velocities of the mitral annulus and basal septum.

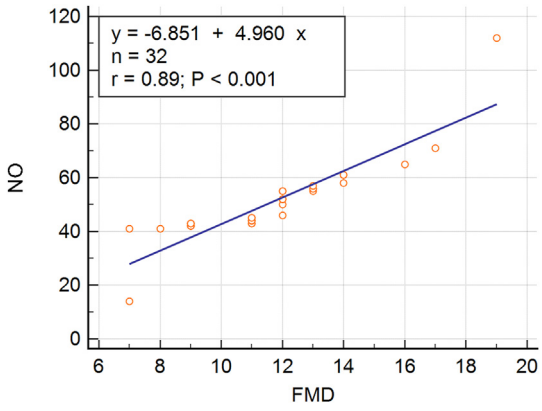

Fig. 4. Scatter plot for illustration of correlation between NO and FMD. Abbreviations: FMD: Flow-mediated dilation, NO: Nitric Oxide.

rate) exceeded 99% (details were included in the supplementary file 1.

5. Discussion

SCD is far from being the sole hemolytic anemia; the prothrombotic state induced by SCD is responsible for multi-organ dysfunction. Neurologic sequelae, leg ulcers, and renal dysfunction are part of the wide spectrum of multiorgan system involvement observed in SCD [11].

In recent years, special focus has been placed on microvascular dysfunction resulting from intravascular hemolysis in the context of SCD. Kaur and colleagues followed in a cohort study of myocardial involvement in SCD disease patients which concluded that cardiac injury occurs in 18 % of patients in the form of elevated troponin and Cardiac Magnetic resonance (CMR) changes consistent with microvascular injury [12].

This goes in agreement with our results; our study evidence of endothelial dysfunction was evident in SCD cases in the form of impaired FMD. This endothelial dysfunction was significantly correlated with each of the measures of LV functions, namely LV EF, LV GLS for systolic function and LV E/E' ratio for LV diastolic dysfunction.

Impaired FMD is not a new finding in SCD. Belhassen et al. tested FMD in a small group of patients with SCD and found that endothelial dysfunction is a hallmark of SCD [13]. The latter findings were confirmed by Zawar et al. and Ayoola et al., who revealed the role of the observed endothelial dysfunction in the induction of renal involvement in SCD patients. However, to the best of our knowledge, our study is the first to correlate endothelial dysfunction as expressed by brachial FMD with myocardial involvement in SCD [14,15].

Coronary circulation might be affected by this state of vascular stiffness, and reduced coronary perfusion can induce progressive ventricular dysfunction as observed in the study. Several autopsies of SCD patients showed evidence of myocardial scarring without frank coronary obstruction, which is strongly suggestive of coronary microvascular dysfunction. Another study conducted on 373 patients with sickle cell disease showed a 32% prevalence of coronary microvascular disease in the study cohort. The mean age of study participants ranged between 19 and 67, while in this study we demonstrated that the process of NO dysregulation and microvascular dysfunction starts at an earlier age in SCD [12].

The endothelial dysfunction observed in SCD has always been related to the consumption of circulating vasodilators because of intravascular hemolysis. The consumption of serum NO has been demonstrated in many previous studies. Vona and colleagues outlined a reduced NO status in patients with SCD. However, to the best of our knowledge, our report is the first to demonstrate a statistically significant relationship between brachial FMD and serum NO levels. The latter relationship, together with the relationship between FMD and measures of left ventricular dysfunction, proves that intravascular hemolysis induces NO deficiency and subsequent microvascular involvement, leading to the observed ventricular dysfunction [16].

6. Conclusion

This article relates biochemical and radiological measures of endothelial function in SCD and outlines the role of microvascular dysfunction as measured by brachial FMD in the induction of left ventricular dysfunction in SCD.

The aggregated evidence in this report could be used to tailor new strategies to delay ventricular dysfunction in SCD patients. These strategies should aim to address the refill of serum NO stores to improve microvascular dysfunction and delay cardiac injury.

Larger studies with a special focus on coronary microvascular dysfunction is needed to ascertain the results of this study and benchmarking echocardiographic results against cardiac magnetic resonance imaging is also needed to improve the credibility of these findings.

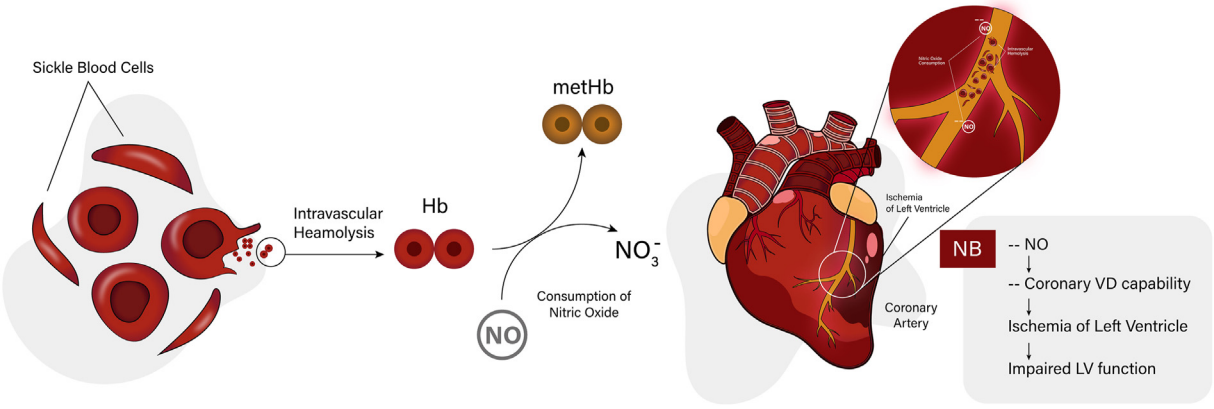

Fig. 5. Our proven hypothesis regarding the main pathophysiologic mechanisms behind LV dysfunction in SCD. Abbreviations: Hb: Hemoglobin, LV: Left ventricle, NO: Nitric Oxide, SCD: Sickle cell disease, VD: Vasodilatation.

Fig. 5 summarizing the pathophysiologic mechanisms, unleashed in this study, that can explain LV dysfunction in SCD cases.

Authors contributions

Conception of the idea and supervision: AA, HA.
Data collection: AA, MH, RA, GH, MAH, MGA, HT, NT, ND, NS, RF, MY, MG, HA.
Drafting of the manuscript: AA, MH, RA, GH, MAH, MGA, HT, NT, ND, NS, RF, MY, MG, HA.
All authors read and approved the final copy of the manuscript.

Data availability

Data will be made available upon reasonable request.

Funding

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Conflict of interest

Authors declare “no conflict of interest.”

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