Association Between Endothelial Dysfunction, Biomarkers of Renal Function and Disease Severity in Sickle Cell Disease

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ABSTRACT

Background: Endothelial dysfunction (ED), as ascertained by brachial artery flow-mediated dilatation (FMD), is a known feature of sickle cell disease (SCD), which is present both in crisis and in steady-state. The assessment of FMD was introduced to examine the vasodilator function. Our objective was to establish the relationship between ED, determined by FMD, and biomarkers of renal dysfunction, and biomarkers of disease severity in SCD subjects asymptomatic of renal disease.

Methods: We enrolled 44 patients with homozygous SCD in steady-state and 33 age- and sex-matched controls between 2013 and 2014 in a tropical tertiary hospital. Ultrasonographic FMD of the right brachial artery, renal arterial Doppler, complete blood count, creatinine, fetal hemoglobin, soluble P-selectin, and Cystatin C (Cys-C) levels were determined. Using the median FMD value of the control group, the SCD subjects were further classified into two groups for comparison.

Results: The median FMD in SCD subjects of 3.44 (IQR: 0.00 – 7.08) was significantly lower than that of controls which was 5.35 (IQR: 3.60 – 6.78), \( P = 0.043 \). There was negative correlation between FMD and Cys-C levels (\( r = -0.372; P = 0.013 \)) along with renal artery resistivity index; RARI (\( r = -0.307; P = 0.042 \)) in SCD subjects. Additionally, Cys-C level was significantly higher in SCD subjects with FMD < 5.35.

Conclusions: Brachial artery FMD was significantly lower in SCD subjects compared to a control group. Cys-C and RARI, show a negative correlation with FMD indicating that renal function is related to ED in SCD.
INTRODUCTION

Sickle cell disease (SCD) is an inheritable genetic disorder of hemoglobin structure with variable clinical manifestations. Annually, about 312,000 people are born with hemoglobin SS genotype worldwide, with up to 236,000 of these in sub-Saharan Africa. 1, 2 Nigeria has the highest disease burden in the world. 3 The prevalence of SCD across sub-Saharan Africa is between 10 and 45%. 4, 5

The vascular endothelium performs endocrine, autocrine, and paracrine functions and is the largest organ in the body (being the one-cell thick innermost layer lining of all blood vessels). 6 It helps to regulate vascular tone, maintain vascular homeostasis, regulate blood flow and constitutes an antithrombotic surface for smooth passage of blood elements/constituents. 6 Endothelial dysfunction (ED) is a known feature of SCD, which is present both in crisis and in steady-state. 7, 8 It has been demonstrated in both children and adults with SCD 8, 9, and is more severe in sickle cell anemia than sickle cell trait patients. 10 Impaired (reduced) sonographic brachial artery flow-mediated dilatation (FMD) is a recognized biomarker for ED in SCD. 11 The assessment of FMD is a noninvasive approach to examining vasodilator function in vivo and has been used as a surrogate marker of vascular health. It can describe any vasodilatation of an artery following an increase in luminal blood flow and internal-wall shear stress. 12

We aimed at evaluating ED in SCD using sonographic brachial artery FMD, compare the FMD in SCD patients to that of controls with HbAA genotype, and to determine any possible association/relationship among the trio of ED, biomarkers of renal function along with indices of SCD severity in patients asymptomatic of renal disease. To the best of our knowledge, no study has shown the relationship between FMD and Cystatin-C (Cys-C) along with renal artery resistivity index (RARI).
MATERIALS AND METHODS

We enrolled 44 homozygous SCD (HbSS) in steady-state based on the criteria defined by Ballas et al,13 attending the hematology clinic of the local institution, along with 33 age and sex-matched controls (HbAA) in this cross-sectional comparative study. The study protocol was approved by the institutional review board, and informed written consent was obtained from all the participants. The genotype of all the participants had been confirmed previously by hemoglobin electrophoresis. We excluded subjects with risk factors for ED such as hypertension, diabetes, obesity, hypercholesterolemia, stroke, and smokers. We also excluded the carrier state for thalassemia (alpha and beta-thalassemia traits) by considering their medical history, full blood count, and the red cell indices (MCV and MCH). None of the subjects was on Hydroxyurea or Nicosan.

Demographic and clinical characteristics

Demographic and clinical data were obtained using a structured data sheet. Systolic and diastolic blood pressures (SBP and DBP, respectively) were measured over the left arm brachial artery region in resting state using an analog mercury sphygmomanometer.

Laboratory Evaluation

An Enzyme-linked immunosorbent (ELISA) using quantitative competitive immunoassays was done to determine fetal hemoglobin (HbF), soluble P-selectin (sP-selectin), kidney injury molecule (KIM-1), homocysteine (HCY) and Cystatin-C (Cys-C) levels according to the manufacturer’s instructions (assay kits were procured from Neobiolab, 245 First Street, 18th floor, Cambridge, Massachusetts 02142, USA). Serum total cholesterol and low-density lipoprotein (LDL) were assessed using cardiochek PA analyser (PTS Diagnostics Headquarters, 7736 Zionsville Road Indianapolis, IN 46268, USA). Hemoglobin concentration (Hb. conc.),
platelet count, white blood cell count (WBC), Peripheral capillary oxygen saturation levels (SpO₂), fasting blood sugar, along with serum creatinine, were assayed. Creatinine assay along with Hb. Conc., WBC, and platelet count weren’t done for the controls. The estimated glomerular filtration rate (eGFR) was done using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation.¹⁴

**Brachial artery sonographic Flow-Mediated Dilatation**

The lead author performed the brachial artery FMD assessment on all the subjects using a Doppler-enabled MINDRAY DC-7 ultrasound scanner (Shenzhen Mindray Bio-medical Electronics, Nanshan, Shenzhen, China) with a 7.5 to 12 MHz linear array transducer. The right brachial artery FMD protocol were the same as described previously.¹⁵,¹⁶ After a 10 – 15 minutes rest at room temperature, the subjects were requested to lie supine, and their right arm was exposed and abducted to 15 degrees. Acoustic gel was applied proximal to the antecubital fossa, and the brachial artery was scanned in a longitudinal section 5 – 10 cm to get a clear view of both walls of the artery. Once an optimal image was obtained, the surface of the skin was marked, and the arm kept in the same position throughout the study. The brachial artery luminal diameter was measured as the distance between the anterior intima-lumen interface to the posterior intima-lumen interface and labeled as D₁. A blood pressure cuff was then applied to the forearm below the elbow and inflated to 50 mmHg above the SBP for 5 minutes to elicit an endothelium-mediated response, and then deflated after 5 minutes of cuff occlusion. The same brachial artery segment underneath the area of the skin marked was interrogated continuously for 30 seconds before and for 90 seconds after cuff deflation. The second arterial diameter (D₂) was taken as the maximum diameter obtained between 60 and 90 seconds after cuff release.
All measurements were obtained during the systolic phase. The average of 3 measurements was used for FMD calculation.

FMD was calculated as the percentage change in diameter after reactive hyperaemia relative to the baseline using the formula:

\[ \text{FMD} = \frac{D_2 - D_1}{D_1} \times 100\% \]

Timing, room temperature, and experience of the examiner could affect the results obtained.\textsuperscript{15}

**Renal arterial Doppler sonography**

Doppler sonography of the right renal artery was done with the subject in the left lateral decubitus position to visualize the artery on greyscale imaging. Spectral Doppler of the interlobar or segmental arteries was then performed with a small sample volume (1 mm). Angle correction was not carried out as only the resistive index (RI), and pulsatility index (PI) are of interest. Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) software version 20 (SPSS Inc., Chicago, IL, USA). Data normality was determined using Kolmogorov Smirnov’s test. Chi-square (\(\chi^2\))/Fisher’s exact test was used to compare proportions, Independent samples Mann-Whitney U test was used to compare the medians, Independent samples t-test was used to compare the means, while Spearman rho was used for correlational analysis. Statistical significance was set at \(P \leq 0.05\).
RESULTS

Forty-four HbSS SCD subjects with a median age of 24.5 years (IQR: 19.5 – 32.0 years) were enrolled along with a control group with a median age of 24.0 years (IQR: 21.0 – 27.0 years). The SCD subjects comprised 23 (52.3%) males and 21 (47.7%) females while the control subjects comprised 17 (51.5%) males and 16 (48.5%) females. No significant difference was noted between the ages of the subjects in both groups (Table 1).

The median FMD in SCD subjects of 3.44 (IQR: 0.00 – 7.08) was significantly lower than that of controls which was 5.35 (IQR: 3.60 – 6.78); \( P = 0.043 \) [Table 1]. No significant gender difference was noted in the FMD among SCD subjects \( (P = 0.981) \) and among control subjects \( (P = 0.790) \).

There was significant modest negative correlation between FMD and serum Cys-C levels \( (r = -0.372; P = 0.013) \) and between FMD and renal artery resistivity index; RARI \( (r = -0.307; P = 0.042) \) (Table 2) in SCD subjects. No significant correlation was noted between FMD and other biomarkers of SCD severity (Table 2), like HbF, sP-selectin, and HCY.

Using a FMD cut-off of 5.35, which is the median value obtained in the control population, SCD subjects were separated into two groups, the median Cys-C levels in SCD subjects with FMD < 5.35 (4.9; IQR = 3.1 – 7.4) was significantly higher than that of SCD subjects with FMD ≥ 5.35 (1.6; IQR = 1.4 – 9.3) (Table 3). A significant difference was also noted in the KIM-1 values of both groups. Although RARI, was slightly higher in SCD subjects with FMD < 5.35 than in those with FMD ≥ 5.35, this was not statistically significant (Table 3).

Among SCD subjects with FMD < 5.35, serum Cys-C levels was significantly higher in those with urine albumin-creatinine ratio (UACR) > 300mg/g (13.4; IQR = 1.6 – 13.4) than in those with UACR 30-300mg/g (6.4; IQR = 5.6 – 10.5) and those with UACR < 30mg/g in that order.
(3.4; IQR = 2.1 – 5.0) (Figure 1) with $P = 0.006$. In this subgroup, there was significant correlation between FMD and UACR ($r = 0.576; P = 0.001$); FMD and serum creatinine levels ($r = 0.418; P = 0.024$); FMD and $eGFR_{\text{CKD-EPI}}^{17}$ ($r = 0.430; P = 0.020$); and between FMD and serum sP-selectin levels ($r = 0.389; P = 0.037$) (Table 4). However, in subjects with FMD $\geq 5.35$, there was no significant difference in serum Cys-C levels based on their UACR values (Figure 1) ($P = 0.531$). In addition, there was no significant correlation between FMD and UACR, serum creatinine levels, $eGFR_{\text{CKD-EPI}}$ or sP-selectin (Table 4).

No significant correlation was observed between Cys-C and RARI in both subjects with FMD $< 5.35$ and those with FMD $\geq 5.35$ (Table 4).
DISCUSSION

Endothelial dysfunction has been implicated in the chronic arterial vasculopathy seen in SCD patients, which is said to be responsible for diverse multi-organ pathologies. \(^{18}\) Ultrasonography has proved to be useful in assessing vasculopathic changes of the cerebral arteries, \(^{19, 20}\) renal \(^{21, 22}\), and femoral arteries \(^{23}\) in SCD. In contrast to these arteries which reflect changes to localized regions of the body, FMD of the brachial artery has the advantage of giving an overview of the overall vascular health of the body.

Several possible aetiologies have been proposed for ED in SCD, including abnormal shear-stress mediated vasodilatation, \(^{24}\) adhesion, and interaction between sickled red blood cells (SRBC) and endothelial cells, SRBC membrane stiffness, hypoxia, nitric oxide deficiency, blood hyperviscosity, and renal function. \(^{10, 25-29}\)

FMD, from this study, was significantly lower in SCD patients than controls. This corroborates with the findings in Indians. \(^{6, 10, 30}\) The mean age of the sample populations were similar to this study (25.39 ± 6.14 years; sample size = 44); Raghuwanshi et al. \(^{30}\) (Male = 24.41 ± 6.59 years; Female = 25.00 ± 7.56 years; sample size = 25), Zawar et al. \(^{10}\) (23.15 ± 5.27 years; sample size = 37), and al-Janabi et al. \(^{6}\) (27.0 ± 8.9 years; sample size = 30). On the other hand, Hadeed et al. \(^{9}\) in France studied 30 younger SCD children (mean age of 12.3 ± 4.5 years) and found no significant difference in FMD between the SCD cases and age and sex-matched controls, concluding that manifestations of ED may be more evident in life as the disease progresses. However, their finding was at variance with that of an earlier French study \(^{8}\) in children (mean age = 10.4 ± 3.3 years; sample size = 21), which reported that FMD was significantly lower in children with sickle cell anemia than in controls and that it did not correlate with age. \(^{8}\) Hadeed et al. \(^{9}\) also reported, as we found in our study, no correlation
between FMD and age. Given the mean age of all these studies, it is conceivable to surmise that increasing age might play a role in the advent of ED in SCD though further studies designed purposely to address that question is desirable.

Regarding the relationship among FMD, RARI, and biochemical parameters of renal function, we observed a statistically significant negative correlation between FMD and Cys-C along with RARI. Furthermore, Cys-C was significantly higher in SCD subjects with FMD < 5.35 than in those with FMD ≥ 5.35. These findings suggest that SCD patients with impaired FMD are more likely to have impaired renal function buttressing similar observations by Tharaux et al and Ataga et al. Albuminuria, an early marker of renal injury, which was used by Ataga, has several causes other than impaired renal function and may not be a reliable marker of renal function compared to the use of Cys-C, which was used in our study. Ataga examined the FMD in 23 SCD subjects with varying degrees of albuminuria and found that UACR and serum endothelin – 1 (ET - 1) level were inversely correlated with FMD. They concluded that their study established an association of albuminuria with ED in SCD and that elevated ET-1, by mediating ED, may be contributory to SCD-related glomerulopathy. We noted a strong positive correlation between UACR and serum Cys-C in subjects with FMD < 5.35 and none in those with FMD ≥ 5.35. To the best of our knowledge, our study would be the first to report a significant relationship between FMD and Cys-C along with RARI in SCD. This finding may imply that as biomarkers of renal function correlate with FMD in SCD while other biomarkers of SCD disease severity do not, monitoring and management of renal function will be critical in SCD in order to preserve FMD and prevent ED. Although RARI is a proven tool for evaluating various renal diseases, it should also be noted that RARI is also an indicator of cardiovascular outcome. In a study by Ike R et al, biochemical and histopathologic
parameters showed statistically significant correlations with RI. However, stepwise multiple regression analysis showed that only atherosclerosis was chosen as an independent risk factor for increased RI. The consistent renal Doppler finding in SCD across board has been a statistically significantly elevated intrarenal arteries RI above those of HbAA controls with a reported a sensitivity of 100% and a specificity of 66.7% for RI > 0.70 in detecting increased resistance within the intrarenal arteries of HbSS patients.\textsuperscript{21,37}

Our study did not demonstrate any relationship between serum creatinine, a biomarker of renal function, and FMD. The discordance in the relationship between Cys-C and creatinine with FMD, relating to glomerular filtration, may be because while Cys-C is freely filtered by the glomerular membrane, it is neither reabsorbed nor secreted in the kidney tubular system unlike creatinine.\textsuperscript{38} Similarly, no relationship was noted between eGFR and FMD. These findings may be because serum creatinine is less sensitive to renal function derangement than serum Cys-C.\textsuperscript{39} Additionally, Coll et al.,\textsuperscript{40} noted that serum creatinine starts to increase above normal values when GFR was 75mL/min/1.73m\textsuperscript{2} in contrast to serum Cys-C which starts to rise at a higher GFR value of 88mL/min/1.73m\textsuperscript{2}. In this study, the median eGFR was 87mL/min/1.73m\textsuperscript{2}.

KIM-1 is released by proximal tubular cells in response to acute ischemic or hypoxic injury to the kidney.\textsuperscript{41} In this study, it was significantly higher in the group with FMD $\geq$ 5.35 than in those with FMD < 5.35. This might reflect acute ischemic (or hypoxic) kidney injury that predates the more chronic ED.

Hypoxia, resulting from chronic anemia, in SCD has also been shown to be a marker of disease severity.\textsuperscript{42,43} Hb. conc., however, didn’t show any significant correlation with FMD (Table 2).
As renal impairment worsens, the prevalence of anemia increases, which affects nearly all patients with Stage 5 CKD. Erythropoietin, which is produced by the kidneys and responsible for stimulation of erythropoiesis, is decreased in CKD and thus accountable for anemia (Figure 2). Sherwood et al. confirmed that SCD patients had reduced erythropoietin levels in comparison to a control population. Tissue ischemia and infarction resulting from vascular occlusion in SCD are primarily due to the microvascular obstruction by SRBC, although vasculopathic changes in larger vessels are also known to affect the kidneys (Figure 2).

Circulating microparticles found in patients with end-stage renal failure have been associated with ED, which is a significant determinant of cardiovascular risk. Likewise, ED has been established in our study and others to be associated with SCD as a result of the endothelial damage and intima hyperplasia. As the disease progresses in SCD and ED worsens along with anemia, renal function depreciates which further compounds on the degree of endothelial function in a vicious cycle (Figure 2).

Vasculopathy in SCD has been well documented, especially regarding the cerebral arteries. As SCD, through its complex pathophysiologic processes, results in arterio-occlusive disease, which could result in renal failure due to ischemia, renal failure could also, in addition, contribute to the compromised blood flow by compromising substantial artery compliance (Figure 2).

Other biomarkers of disease severity in SCD subjects (Table 2) didn’t show any significant relationship with FMD. HbF is the most potent modulator of the clinical and hematologic features in SCD. Higher HbF levels were associated with a reduced rate of acute painful episodes, fewer leg ulcers, along with reduced SCD severity. Endothelial sP-selectin
plays a crucial role in leukocyte recruitment as well as the adhesion of SRBC to the endothelium, ultimately leading to an impairment of microvascular circulation involved in the development of painful vaso-occlusion. 52-54 HCY, which is elevated in SCD in this study (Table 1) and other studies 55, is a strong risk factor for atherosclerotic disease in the peripheral arteries along with arterial thromboembolism. 56 HbF, sP-selectin, and HCY showed no correlation with FMD (Table 2).

Only homozygous SCD subjects were recruited in this study in steady state. Further studies in heterozygous SCD and also in SCD subjects in vaso-occlusive crisis would add to the body of knowledge regarding FMD in the disease.

In conclusion, brachial artery FMD is an essential test in the management of SCD patients for non-invasive assessment of the vascular endothelium status. There is a relationship between FMD, RARI, and Cys-C in SCD patients such that impairment of FMD could also be a proxy marker for the onset of renal impairment in this group of patients. Even though our findings show relationships rather than causation, we believe it is still a step forward in the ongoing quest to unravel the mysteries of this genetic disease. Determining the exact age at which FMD impairment sets in children with SCD could be the subject of a future study.
DISCLOSURES

The authors have no relevant conflicts to disclose.

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AUTHOR CONTRIBUTIONS

Oluwagbemiga Ayoola: Conceptualization; Investigation; Methodology; Project administration; Writing - original draft; Writing - review and editing

Rahman Bolarinwa: Investigation; Methodology; Writing - original draft; Writing - review and editing

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Table 1. Characteristics of SCD subjects and controls

| Variables         | SCD n = 44 | Controls n = 33 | P value |
|-------------------|------------|----------------|---------|
| Age, years        | 24.5 (19.5 – 32.0) | 24.0 (21.0 – 27.0) | 0.305*# |
| Height, m         | 1.6 (1.6 – 1.7) | 1.6 (1.5 – 1.7) | 0.896*# |
| Weight, kg        | 49.1 ± 10.0 | 61.3 ± 12.3 | <0.001** |
| BMI, kg/m²        | 18.1 (16.8 – 20.4) | 21.6 (19.9 – 25.7) | <0.001*# |
| SpO², %           | 95.0 (92.0 – 98.0) | 98.0 (96.0 – 98.5) | <0.001*# |
| Hb conc. g/dl     | 8.3 (7.3 – 8.7) |                   |         |
| WBC, 10³/μL       | 9.5 (7.3 – 11.7) |                   |         |
| Platelet, 10³/μL  | 295.5 (192.0 – 367.5) |               |         |
| HbF, ng/ml        | 373.3 (316.8 – 448.28) | 273.9 (212.2- 335.7) | <0.001*# |
| sP-Selectin, ng/ml| 78.7 (68.9 – 84.0) | 72.7 (67.1 – 78.5) | 0.007*# |
| HCY, µmol/l       | 16.4 (10.1 – 28.8) | 8.3 (5.0 – 19.3) | 0.008*# |
| Cys-C, mg/l       | 4.8 (1.6 – 7.9) | 1.1 (0.5 – 1.6) | <0.001*# |
| KIM-1, pg/ml      | 445.0 (320.0 - 623.0) | 125.0 (120.0 – 184.0) | <0.001*# |
| Creatinine, µmol/l| 87.5 (64.5 – 124.3) |                   |         |
| eGFR<sub>CKD-EPI</sub>, mL/min per 1.73m² | 87.5 (59.0 – 118.8) |                   |         |
| UACR, mg/g n (%)  | <30 26 (59.1) | 33 (100) | <0.001* |
|                  | <30 - 300 14 (31.8) | 0 (0) |         |
|                  | >300 4 (9.1) | 0 (0) |         |
| RARI             | 0.70 (0.66 – 0.72) | 0.59 (0.54 – 0.64) | <0.001*# |

*Chi square (χ²)/Fisher’s exact test statistic was used to compare proportions.

#Independent samples Mann-Whitney U test was used to compare the medians.

**Independent samples t-test was used to compare the means.

BMI – body mass index; SpO² - Oxygen saturation; Hb Conc. - Hemoglobin concentration; WBC - White blood cell count HbF – Hemoglobin F; sP-Selectin – soluble P-selectin; Cys-C – cystatin C; KIM-1 – Kidney injury molecule-1; eGFR<sub>CKD-EPI</sub> – Glomerular filtration rate Chronic Kidney Disease Epidemiology Collaboration; UACR - urine albumin-creatinine ratio; RARI – Renal artery resistivity index.
|                  | SCD Subjects |                   | Control Subjects |                   |
|------------------|--------------|-------------------|------------------|-------------------|
|                  | Correlation  | p value           |                  |                  |
|                  | Coefficient* |                   |                  |                  |
| Age, years       | 0.153        | 0.322             | 0.079            | 0.311            |
| Height, m        | 0.117        | 0.450             | -0.210           | 0.294            |
| Weight, kg       | -0.063       | 0.683             | -0.179           | 0.372            |
| BMI, kg/m²       | -0.088       | 0.571             | 0.014            | 0.943            |
| SpO₂, %          | 0.127        | 0.410             | 0.147            | 0.416            |
| Hb conc, g/dl    | 0.125        | 0.418             |                  |                  |
| WBC, 10³/μL      | 0.077        | 0.620             |                  |                  |
| Platelet, 10³/μL | -0.182       | 0.238             |                  |                  |
| HbF, ng/ml       | 0.011        | 0.945             | 0.114            | 0.528            |
| sP-Selectin, ng/ml | -0.182     | 0.238             | 0.003            | 0.988            |
| HCY, μmol/l      | -0.204       | 0.184             | -0.022           | 0.905            |
| Cys-C, mg/l      | -0.372       | **0.013**         | 0.020            | 0.912            |
| KIM-1, pg/ml     | 0.179        | 0.245             | 0.156            | 0.384            |
| Creatinine, μmol/l | -0.166     | 0.281             |                  |                  |
| eGFR<sub>CKD-EPI</sub>, mL/min per 1.73m² | 0.111 | 0.473             |                  |                  |
| UACR             | -0.074       | 0.632             | -0.146           | 0.416            |
| RARI             | -0.307       | **0.042**         | -0.017           | 0.923            |

BMI – body mass index; SpO₂ - Oxygen saturation; Hb conc.- Hemoglobin concentration; WBC- White blood cell count; HbF – Fetal Hemoglobin; sP-Selectin – soluble P-selectin; Cys-C – cystatin C; KIM-1 – Kidney injury molecule-1; UACR - urine albumin-creatinine ratio; eGFR<sub>CKD-EPI</sub> – Glomerular filtration rate<sup>Chronic Kidney Disease Epidemiology Collaboration</sup>; RARI – Renal artery resistivity index
Table 3. Categorization of SCD subjects based on FMD

| Variables            | SCD FMD < 5.35 | SCD FMD ≥ 5.35 | P value  |
|----------------------|----------------|----------------|----------|
|                      | n = 29         | n = 15         |          |
| Gender, n (%)        |                |                |          |
| Male                 | 14 (48.3)      | 9 (60.0)       | 0.535*   |
| Female               | 15 (51.7)      | 6 (40.0)       |          |
| Age, years           | 24.0 (19.0 – 31.5) | 26.0 (21.0 – 32.0) | 0.637°   |
| Height, m            | 1.6 (1.5 – 1.7) | 1.6 (1.6 – 1.8) | 0.102°   |
| Weight, kg           | 48.5 ± 11.1    | 50.3 ± 7.7     | 0.590**  |
| BMI, kg/m²           | 18.1 (16.6 – 20.5) | 17.7 (16.8 – 20.2) | 0.785°   |
| SpO₂, %              | 94.0 (89.0 – 97.5) | 95.0 (93.0 – 98.0) | 0.178°   |
| Hb conc, g/dl        | 8.1 ± 1.3      | 8.3 ± 1.1      | 0.499**  |
| WBC, 10³/μL          | 9.9 ± 4.1      | 10.0 ± 3.8     | 0.918**  |
| Platelet, 10³/μL     | 302.9 ± 148.6  | 274.7 ± 146.7  | 0.552**  |
| HbF, ng/ml           | 383.6 (328.8 – 444.5) | 349.3 (260.3 – 472.6) | 0.421°   |
| sP-Selectin, ng/ml   | 79.4 (65.9 – 84.8) | 78.5 (76.0 – 82.5) | 0.921°   |
| HCY, μmol/l          | 18.7 (11.9 – 32.7) | 14.4 (7.4 – 25.6) | 0.360°   |
| Cys-C, mg/l          | 4.9 (3.1 – 7.4)  | 1.6 (1.4 – 9.3) | 0.039°   |
| KIM-1, pg/ml         | 420.0 (297.5 – 540.0) | 560.0 (380.0 – 798.0) | 0.042°   |
| Creatinine, μmol/l   | 90.0 (69.0 – 105.5) | 78.0 (49.0 – 128.0) | 0.766°   |
| eGFR<sub>CKD-EPI</sub> mL/min per 1.73m² | 87.0 (60.5 – 115.0) | 92.0 (58.0 – 146.0) | 0.692°   |
| RARI                 | 0.70 (0.67 – 0.73) | 0.69 (0.62 – 0.71) | 0.160°   |

*Chi square (χ²)/Fisher’s exact test statistic was used to compare proportions. Independent samples Mann-Whitney U test was used to compare the median.; **Independent samples t-test was used to compare the means. BMI – body mass index; SpO₂ - Oxygen saturation; Hb conc.- Hemoglobin concentration; WBC- White blood cell count; HbF – Fetal Hemoglobin; sP-Selectin – soluble P-selectin; Cys-C – cystatin C; KIM-1 – Kidney injury molecule-1; eGFR<sub>CKD-EPI</sub> – estimated Glomerular filtration rate Chronic Kidney Disease Epidemiology Collaboration.; RARI – Renal artery resistivity index.
Table 4. Spearman correlation of Cys-C with other variables in the 2 SCD subject groups

|                  | UACR, mg/g | Creatinine, µmol/l | GFR, mL/min per 1.73m² | sP-Selectin, ng/ml | RARI | KIM-1, pg/ml |
|------------------|------------|--------------------|------------------------|-------------------|------|-------------|
| Subjects with FMD < 5.35 |            |                    |                        |                   |      |             |
| Spearman’s Rho   | 0.576      | 0.418              | 0.430                  | 0.389             | 0.129| 0.032       |
| P value          | **0.001**  | **0.024**          | **0.020**              | **0.037**         | 0.506| 0.868       |
| Subjects with FMD ≥ 5.35 |     |                    |                        |                   |      |             |
| Spearman’s Rho   | -0.232     | -0.268             | 0.352                  | -0.396            | -0.059| 0.566       |
| P value          | 0.406      | 0.335              | 0.198                  | 0.144             | 0.834| **0.028**   |

Cys-C – cystatin C; UACR - urine albumin-creatinine ratio; eGFR – Estimated Glomerular filtration rate; sP-Selectin – soluble P-selectin; RARI- Renal artery resistivity index; KIM-1- Kidney injury molecule-1
Figure 1: Clustered Box Plot showing the distribution of Cystatin-C levels in SCD subjects based on FMD (flow-mediated dilatation) category and UACR (urine albumin creatinine ratio) category.
Figure 2: A chart showing the relationship between renal impairment and SCD pathophysiologic processes.