

Transthoracic Echocardiography and 6-Minute Walk Test in Kuwaiti Sickle Cell Disease Patients

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Key Words

Hemoglobinopathies · Sickle cell disease · Tricuspid regurgitant jet velocity · Echocardiography · Pulmonary hypertension

Abstract

Objective: The aim of this study was to investigate cardiac abnormalities in Kuwaiti sickle cell disease (SCD) patients using markers such as tricuspid regurgitant jet velocity (TRJV), pulmonary artery systolic pressure (PASP), and the 6-minute walk (6MW) test and correlate these findings with clinical, hematological, and biochemical parameters. **Materials and Methods:** Seventy-three patients with SCD and 70 matched controls were studied. The cardiac status was investigated using transthoracic echocardiography in 57 patients; the 6MW test was carried out in patients and controls. Complete blood counts and hemolytic parameters were assessed. **Results:** Reticulocytes, bilirubin, and lactate dehydrogenase were significantly higher ($p < 0.0001$) in patients, while hemoglobin (Hb) and haptoglobin were lower ($p < 0.0001$) than in controls. The mean fetal Hb among patients was $15.85 \pm 8.7\%$. Of the 57 patients, 14 (24.5%) and 15 (26%) had mild tricuspid and mitral regurgitation, respectively. The

mean ejection fraction, TRJV, and PASP were $63.9 \pm 6.3\%$, 1.7 ± 0.5 m/s, and 23.0 ± 7.3 mm Hg, respectively. Three (5.2%) patients had mildly raised TRJV (2.6–2.97 m/s, normal range < 2.5 m/s) while 8 (14%) had high PASP (mean 35.3 ± 5.1 mm Hg, normal range < 30 mm Hg). Hb, hematocrit, and reticulocytes were different ($p = 0.010$, $p = 0.006$, and $p = 0.011$, respectively) between patients with normal and high PASP. All 3 patients who had a high TRJV had a high PASP, and 2 of these patients died during follow-up. The systolic and diastolic blood pressure, oxygen saturation before and after the 6MW test, and distance walked were lower ($p = 0.006$, $p = 0.000$, $p = 0.002$, $p = 0.000$, and $p = 0.000$, respectively) in patients compared to controls. **Conclusion:** Raised PASP was common in Kuwaiti SCD patients while raised TRJV was not.

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Introduction

Pulmonary vasculopathy is associated with pulmonary hypertension (PHT) in sickle cell disease (SCD), contributing significantly to its mortality, especially as more patients survive into adulthood. It has been shown that mortality due to PHT occurs at a higher rate in SCD pa-

tients compared to controls and with a lower degree of PHT [1]. The pathophysiology of this disease is related to the nitric oxide (NO) scavenging that follows the release of cell-free hemoglobin (Hb) from hemolysis. This leads to vasoconstriction, platelet activation, and upregulation of multiple adhesion molecules. The release of arginase further compromises the available substrate for the production of NO in endothelial cells. Repeated episodes of these hypoxic events are characterized by ischemia-reperfusion injury with progressive tissue damage, an altered pulmonary vascular tone, and vascular proliferation in the arterial smooth muscle wall. Eventually, obliterative pulmonary vasculopathy occurs with PHT.

Right-sided cardiac catheterization is the gold standard for the diagnosis of PHT [2], but it is an invasive procedure. Echocardiographic indices of PHT are based on changes such as right ventricular dilatation, paradoxical motion of the ventricular septum, peak tricuspid valve regurgitant jet velocity (TRJV), and pulmonary artery systolic pressure (PASP). The reliability of this echocardiographic technique has been shown in selective cardiac catheterizations comparing the two techniques in patients with SCD [1]. PASP can be estimated noninvasively using Doppler techniques, and when an acceptable TRJV is obtained these estimates show excellent correlation with invasively determined PASP [3].

The 6-minute walk (6MW) test is an objective evaluation technique for functional exercise capacity and it is also useful as a noninvasive screening test for PHT. It reflects the functional exercise level for daily physical activities [4]. This test has also been used as a one-time measure of the functional status of SCD patients, as well as a predictor of morbidity and mortality [5]. Postwalk dyspnea and fatigue levels estimated using the Borg scale are recorded and the total distance a patient can walk in 6 min is calculated [4].

Although SCD is a monogenic disease, it exhibits considerable phenotypic variability and its expression is modified by several other genetic factors, the most important of which include the β -globin cluster haplotype, the α -globin genotype, and the fetal Hb (HbF) level. SCD is relatively common in Kuwait due to a preponderance of consanguineous marriages [6], but it is relatively mild among Kuwaiti patients because they carry the Arab/India β^S haplotype, which is associated with a high Hb F level [7]. Many of the complications [stroke, splenic dysfunction, leg ulcers, priapism, and acute chest syndrome (ACS)], except avascular necrosis and regional brain perfusion deficit, are not common in these patients [8, 9]. Silent brain infarcts have been shown in only about 3% of

children aged <16 years but in up to 20% of adults [10]. Moreover, the frequency of the α -thalassemia trait is about 30% [11]. There has, however, not been any previous study of PHT in this group of patients. The aim of this study was to investigate cardiac abnormalities in Kuwaiti SCD patients using parameters such as TRJV, PASP and the 6MW test as indirect markers of PHT and correlate the results with clinical, hematological, and biochemical parameters.

Materials and Methods

Patients and Controls

For this case control study, consecutive SCD patients (HbSS or $S\beta^0$ -thalassemia) presenting between 2009 and 2011 to the Hematology Clinic, Mubarak Al Kabeer Hospital, Kuwait, were studied. Seventy-three SCD patients (37 males and 36 females) and 70 (36 males and 34 females) age- and sex-matched controls were studied. The Hb genotype was confirmed by high-performance liquid chromatography. The inclusion criterion was patients in stable condition, i.e. without acute illness or a crisis in the preceding 4 weeks. Histories of hypertension, diabetes mellitus, smoking status, and asthma were documented. Informed voluntary consent was obtained from all study participants. Age- and sex-matched, apparently healthy controls were recruited from volunteers including workers in the medical school and among the patients' friends and relatives. This study was approved by the Institutional Ethics Committee and was carried out in accordance with the Helsinki Declaration of 1975, as revised in 2008 [12].

Clinical Data

Age, sex, height, weight, and blood pressure were obtained from patients and controls. The following parameters were documented for the patients: frequency of vaso-occlusive crises and hospital admissions, blood transfusions, ACS, history of splenectomy, leg ulcers, stroke, and treatment with hydroxyurea.

Patients were classified into 2 groups, i.e. severe and nonsevere, depending on how frequently they had vaso-occlusive crises. Those who were hospitalized on more than 3 occasions or who had more than 6 emergency room visits for pain in a year were classified as severe. At least 1 episode of ACS was considered a positive history of ACS.

Laboratory Data

Blood was obtained from both patients and controls by venipuncture in a fasting state. A complete blood count, including reticulocytes and red blood cell indices, was performed on an automated hematology analyzer (Beckman-Coulter LH 750; Beckman-Coulter, Brea, Calif., USA) and the Hb analysis was done using a cation exchange high-performance liquid chromatograph (Bio-Rad VARIANT II Hemoglobin Testing System; USA). Fasting blood sugar, renal function tests (electrolytes, urea, and creatinine) and liver function tests (total bilirubin, alanine transaminase, alkaline phosphatase, γ -glutamyltransferase, total protein and albumin) as well as lactate dehydrogenase (LDH) were determined using a Beckman DXi automated analyzer (Beckman-Coulter). Haptoglobin was determined using Beckman Access/Array (Beckman-Coulter).

Echocardiography

Transthoracic echocardiography was performed only in the 57 patients who agreed to undergo the test. Two-dimensional M mode and color Doppler images were obtained using Vivid-7 Cardiovascular Ultrasound System (GE Healthcare, Milwaukee, Wisc., USA). Echocardiographs were reviewed by a cardiologist (M.Z.). Cardiac dimensions were measured according to American Society of Echocardiography criteria [13].

Heart chamber dimensions, left ventricular posterior wall and interventricular septal thickness, ventricular function, left ventricular stroke volume, valve structure, and abnormal flow across the valves were recorded as previously described [14]. The TRJV was assessed in the parasternal right ventricular inflow, parasternal short-axis, and apical four-chamber views. Continuous-wave Doppler sampling of the peak TRJV was used to estimate the right-ventricular-to-right-atrial systolic pressure gradient with the modified Bernoulli equation [15]. For the purpose of analysis, PHT was defined as a peak TRJV of ≥ 2.5 m/s as previously described.

The pulmonary artery pressure was estimated by measuring the TRJV using continuous-wave Doppler ultrasound. The PASP was quantitated by adding the Bernoulli-derived pressure gradient to the estimated mean right atrial pressure. The mean right atrial pressure was calculated according to the degree of collapse of the inferior vena cava with inspiration: 5 mm Hg for a collapse of at least 50% and 15 mm Hg for a collapse of less than 50% [16]. PASP ≥ 30 mm Hg was considered significantly high in our patient population.

6MW Test

The 6MW test was performed indoors, along a flat, straight, enclosed corridor with a hard surface. The walking course was 30 m in length [17]. Postwalk dyspnea and fatigue levels were recorded according to Borg's scale and the total distance walked was calculated. The transcutaneous arterial oxygen saturation was determined by pulse oximetry (BCI International, Waukesha, Wisc., USA) in the same setting.

Statistical Analyses

The statistical analysis was performed using IBM SPSS (Statistical Package for the Social Sciences) statistical software, version 19 (Chicago, Ill., USA). Data are presented as means \pm SD. Measurements were compared using Student's *t* test. Spearman's rank correlation coefficient was used to investigate the relation between various parameters. $p < 0.05$ was considered statistically significant. Regression analysis was used to identify the predictors of PASP in SCD patients.

Results

Of the 73 patients, 44 (60.3%) and 29 (39.75%) had mild and severe courses of SCD, respectively. Four (5.4%) had a history of ACS. None of the patients had a history of stroke or leg ulcers. Thirteen (17.8%) patients were on hydroxyurea and 60 (82.2%) were on no medication. The reticulocyte count, total bilirubin, and LDH levels were

Table 1. Echocardiographic findings in 57 SCD patients

Interventricular septum diameter, mm	9.6 \pm 0.9 (9 \pm 1)
Left ventricular internal diastolic dimension, mm	48.9 \pm 4.8 (49 \pm 4)
Left ventricular posterior wall dimension, mm	9.5 \pm 1.0 (8 \pm 1)
Left ventricular internal systolic dimension, mm	31.8 \pm 4.4 (30 \pm 5)
Left atrial diameter, mm	34.5 \pm 4.6 (39 \pm 5)
Ejection fraction, %	63.9 \pm 6.3 (58 \pm 7)
TRJV, m/s	1.7 \pm 0.5 (<2.5)
PASP, mm Hg	23.0 \pm 7.3 (<30)

Values are presented as means \pm SD. Values in parentheses are normal ranges.

Table 2. Echocardiographic abnormalities in SCD patients

Tricuspid regurgitation	14 (24.6)
Paradoxical septal motion	0
Mitral valve regurgitation, mild	15 (26.3)
Dilated left ventricle	1 (1.8)
Left ventricular wall hypertrophy	1 (1.8)
Dilated left atrium	0
Dilated right ventricle	0
Dilated right atrium	0
Aortic valve regurgitation	1 (1.8)
Mitral valve leaflet prolapse (anterior)	6 (10.5)
Mitral valve leaflet prolapse (posterior)	1 (1.8)
Mitral valve leaflet prolapse (anterior + posterior)	1 (1.8)

Values are presented as n (%).

significantly ($p < 0.0001$) higher in patients compared to controls, while Hb and haptoglobin levels were significantly ($p < 0.0001$) lower in the former. Of the 73 patients, 51 (69.9%) had HbSS while 22 (30.1%) had $S\beta^0$ -thalassemia. The mean Hb F level was 15.85% for the whole group; 2 (3%) patients had Hb F levels $< 5\%$, while 18 (25%) had levels $> 20\%$ regardless of whether or not they were receiving hydroxyurea therapy. Blood transfusion was rarely given to the patients, except perioperatively or, as in one patient, as a therapeutic modality for chronic priapism. The frequency of blood transfusions correlated negatively with the level of HbF ($r = -0.458$, $p < 0.0001$).

Table 3. Significant parameters in SCD patients with high and normal PASP

	PASP <30 mm Hg (n = 49)	PASP ≥30 mm Hg (n = 8)	p value
RBC, ×10 ¹² /l	3.96±0.81	3.44±0.40	0.011
Hb, g/l	107.13±15.20	93.13±11.34	0.010
Hct, l/l	0.32±0.05	0.28±0.03	0.006
Reticulocytes, %	4.91±2.12	6.91±1.32	0.011

Values are presented as means ± SD. RBC = Red blood cell count.

Table 4. 6MW test

	Patients	Controls	p value
Systolic blood pressure, mm Hg	113.95±11.18	120.23±14.06	0.006
Diastolic blood pressure, mm Hg	66.40±7.86	73.08±9.56	0.000
Distance walked in 6 min, m	384.63±73.07	531.76±83.92	0.000
Heart rate at baseline, bpm	88.72±12.37	84.48±12.68	0.060
Heart rate at the end of the test, bpm	109.90±18.79	115.02±19.19	0.133
Oxygen saturation at baseline, %	96.92±2.36	98.00±1.20	0.002
Oxygen saturation at the end of the test, %	96.15±2.72	97.88±1.44	0.000

Values are presented as means ± SD.

Echocardiography

The measured parameters of echocardiography and the frequencies of abnormal findings are shown in tables 1 and 2. Of the 57 patients who underwent an echocardiographic study, 14 (24.5%) had mild tricuspid regurgitation while 15 (26%) had mild mitral valve regurgitation, which was the most common abnormality. The mean ejection fraction of the study group was 63.9 ± 6.3%. The mean TRJV was 1.7 ± 0.5 m/s. Three (5.2%) patients (all HbSS) had mildly raised TRJV (2.6–2.97 m/s). Of these 3 patients, 2 died 10 months and 3 years after the test, respectively. Both had nonsevere vaso-occlusive crises. The first patient, a 23-year-old morbidly obese (BMI 60.6) female with diabetes and hypertension, had low Hb levels, a high reticulocyte count and transfusion requirement;

her Hb F level was 4% and her oxygen saturation was 98%. The second patient, a 66-year old female diabetic with breast cancer, had similar enhanced hemolytic parameters. Her oxygen saturation was 90% at baseline and dropped to 86% after the 6MW test. Both patients had high Hb F levels (>15%) before treatment with hydroxyurea. If the first patient is considered an outlier because of a very high BMI, the age of the patients with high TRJV was significantly higher than that of patients with TRJV <2.5 m/s (p = 0.005). The third patient with a high TRJV had severe vaso-occlusive crises and was receiving maintenance therapy with hydroxyurea.

The mean PASP of the patients was 23.0 ± 7.3 mm Hg. Eight (14%) patients, including the 3 with TRJV ≥2.5 m/s, had high PASP (≥30 mm Hg); the mean was 35.3 ± 5.1 mm Hg. Hb and hematocrit (Hct) were significantly lower in patients with high PASP, while reticulocyte count was significantly higher (table 3). Binary logistic regression analysis showed that lower Hb levels were significantly associated with a high PASP (OR 0.928, 95% CI 0.870–0.990; p = 0.023). The 57 patients had normal right ventricular size and none had paradoxical septal motion.

6MW Test

The systolic and diastolic blood pressure, oxygen saturation before and after 6MW test, and distance walked were significantly lower in SCD patients compared to controls (table 4). The 8 patients with high PASP had a mean 6MW distance of 393.8 ± 89.7 m compared to 380.7 ± 69.8 m in patients with normal PASP, which was not significantly different (p = 0.706).

Discussion

In the present study, the clinical manifestations in the group of SCD patients were mild to moderate in relation to the frequencies of vaso-occlusive crises, hospital admissions, and blood transfusions. None of the patients had a history of stroke or leg ulcers. However, there was laboratory evidence of chronic hemolysis with low Hb and Hct and high reticulocyte counts, LDH, and bilirubin in comparison to controls.

Although the gold standard for the diagnosis of PHT is cardiac catheterization, a TRJV ≥2.5 m/s correlates well with the diagnosis [18]. While the precise prevalence of PHT is unknown, reported estimates range from 5 to 30% in adult SCD patients [19–21]. In a study by Gladwin et al. [1], PHT reflected by the TRJV was estimated to be present in 32% of patients with SCD, while other studies

have yielded rates of 30–56% [22, 23]. In the present study, the mean TRJV was 1.7 m/s compared to 2.7 m/s in a recent large prospective multicenter study of American patients [24]. Strikingly, only 3 (5.2%) of the Kuwaiti patients included in this study had TRJV values ≥ 2.5 m/s, and 2 of them died 10 months and 3 years after the study, respectively. No other deaths had been reported in the cohort at the time of reporting. The fact that patients with high PASP had significantly impaired hemolytic indices (low Hb and Hct and higher reticulocyte counts) is consistent with the realization that hemolysis aggravates vasculopathy and predisposes to PHT [18]. Both mortalities in this cohort occurred soon after documentation of a raised TRJV. However, this is consistent with the rarity of hemolysis-driven chronic vasculopathy among patients with raised Hb F levels in whom the vaso-occlusive phenotype is more common such that avascular necrosis is the major complication encountered [8].

The use of echocardiography to estimate PASP has been validated in patients with SCD and it correlates well with values obtained by right heart catheterization [1]. Eight (14%) patients (including the 3 with a high TRJV) had high PASP, i.e. ≥ 30 mm Hg, that correlated with the severity of anemia. The 6MW test is a surrogate for functional capacity, or exercise tolerance, and it correlates well with the elevation of pulmonary artery pressure and pulmonary vascular resistance [25]. When interpreting this test in SCD, certain noncardiopulmonary factors such as concurrent pain and avascular necrosis of the hip must be

considered as these can interfere with the performance of the test. This may explain why our patients with high PASP in the present study had 6MW distance not significantly different from the distance walked by patients with normal PASP. However, when patients were compared to controls they had significantly lower 6MW distance.

The patients in this study had some degree of hemolysis, as shown by the relatively high levels of reticulocytes and LDH, and appropriate efforts should be made to identify those at risk for associated complications. Since PHT is a poor prognostic indicator of SCD, it would appear that PASP and TRJV might be noninvasive tests that can be used for screening Kuwaiti patients with SCD.

Conclusion

Raised PASP was common in the Kuwaiti patients with SCD while raised TRJV was not. Hence, the value of PASP as an earlier indicator of poor prognosis needs to be evaluated further.

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