

Clinical and Laboratory Predictors Of Elevated TRV In Sickle Cell Anaemia

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Abstract

Pulmonary hypertension (PH) has become an increasingly recognized complication in sickle cell anaemia (SCA) and is a major cause of morbidity and mortality. Though the burden of SCA in sub-Saharan Africa is high, there is paucity of data on SCA-associated PH with little or no attention given to it in routine patient care. The current study therefore sought to determine the prevalence of PH and its associated risk factors among adult patients with SCA. This was a cross-sectional study involving 76 clinically stable, hydroxyurea-naive participants. We obtained socio-demographic and clinical history. Measurement of Tricuspid Regurgitant jet Velocity (TRV) was obtained via transthoracic echocardiography and lung function was assessed using spirometry and pulse oximetry. Other investigations were complete blood counts, free plasma haemoglobin, serum urea and creatinine. Twenty-five (32.9%) of study participants had elevated TRV (≥ 2.5 m/s) on Doppler echocardiography, which was suggestive of raised pulmonary artery systolic pressure. There were significant associations between elevated TRV and steady-state haemoglobin ($p < 0.001$), blood urea level ($p = 0.030$), presence of chronic leg ulcers ($p = 0.043$) and oxygen saturation ($p < 0.001$) and these may be identifiable and modifiable risk factors for selective screening with echocardiography in a resource poor setting.

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Introduction

Pulmonary involvement in sickle cell disease (SCD) has been shown to be a major factor affecting morbidity and mortality, being recognized also as an important determinant of survival^{1,2}. This has become apparent in recent times as increased numbers of SCD patients reach adulthood due to improved survival strategies in childhood³. The lungs are common sites of hypoxic and ischaemic injury, emboli from marrow infarcts/fat necrosis with increased propensity to developing pneumonias⁴. Chronic lung complications in sickle cell disease describes a state of permanent damage to the lung parenchyma and vasculature. Manifestations include interstitial abnormalities/fibrosis, restrictive lung disease and pulmonary hypertension in its severe form⁵. Emerging evidence have consistently shown that pulmonary hypertension (PH) complicated by right heart failure is a major cause of mortality among adult patients with disease, particularly those with homozygous disease, sickle cell anaemia (SCA)⁶⁻⁸. Pulmonary hypertension is usually asymptomatic in the early stages but may be characterized by dyspnoea on exertion, hypoxaemia, progressive worsening of pulmonary function tests (PFTs) and right heart failure when severe⁹. Even in advanced stages, the elevation in pulmonary artery systolic pressures (PASP) has been shown to be mild to moderate in sickle cell patients compared to those with idiopathic or scleroderma associated PH, yet the former presents with equivalent symptoms of severe dyspnoea, exercise limitation, hypoxaemia and progressive right heart failure¹⁰. This spells out the need for screening and early identification of elevations in PASP using non-invasive means such as trans-thoracic echocardiography (ECHO) instead of invasive right heart catheterization (RHC), which is the diagnostic procedure. This is a validated tool for detecting early rise in pulmonary artery pressure, with PH being more likely if the tricuspid regurgitant jet velocity (TRV) is $\geq 2.5\text{m/s}$. The prevalence of elevated TRV using echocardiography is estimated to be 25-30% among SCA patients and 10-25% in those with genotype SC^{6,8,11}. However, studies have reported that only about 6-11% of patients with TRV $\geq 2.5\text{m/s}$ on ECHO have confirmed pulmonary hypertension on right heart catheterization^{12,13}. Notwithstanding, elevated

TRV $\geq 2.5\text{m/s}$ in sickle cell patients has been significantly associated with decreased exercise tolerance and increased mortality estimated to be about 40% at 40 months of follow-up and is therefore considered an independent risk factor for death among these patients^{6,14,15}. According to the official American Thoracic Society (ATS) practice guideline¹⁶, mortality risk of sickle cell patients can be determined non-invasively by measurement of TRV via doppler echocardiography. The current study sought to determine whether elevated TRV is an identifiable risk factor among our SCA cohort and if present determine associated clinical and laboratory risk factors.

Methods

Study Design

This was an analytical cross-sectional study involving 80 hydroxyurea-naive adult SCA participants, 18yrs and above, who were in their steady state and consented to participate in the study. The study was carried out within the period of March – April, 2014.

Study Site

This study was conducted at the Ghana Institute of Clinical Genetics which renders outpatient and day care services with referrals from other healthcare facilities all over Ghana. It has the largest adolescent and adult sickle cell clinic in Ghana, with over 25,000 registered sickle cell patients in its database. Averagely the clinic attends to 40 patients daily.

Data Collection

Sickle cell anaemia patients with clear documentation of results of electrophoresis in their clinic notes were selected by systematic random sampling using the daily clinic attendance list and screened for eligibility. Four (4) patients were excluded from analysis due to our inability to measure the TRV jet. Patients needed to be in their steady state as acute crisis could cause temporary rise in TRV values and lower lung volumes on spirometry¹⁷. A structured and pre-tested questionnaire was used to obtain socio-demographic and clinical information on all participants. A modified version of St. George's Respiratory questionnaire was used to screen participants for chronic chest diseases and excluded if present. Blood pressure, pulse rate and oxygen saturation were recorded at rest. A general and

cardio-respiratory examination were performed on all participants to further exclude those with respiratory and cardiovascular diseases that are capable of influencing ECHO and spirometry results of the stable participants. Blood samples for complete blood count, blood urea, creatinine and free plasma haemoglobin were taken. Spirometry was performed according to ATS protocol using SCHILLER SPIROVIT SP-1 (Schiller-AG, Switzerland)¹⁸. Forced vital capacity (FVC) and forced expiratory volume per second (FEV₁) were measured.

Trans-thoracic doppler echocardiography was performed for all participants using a Toshiba brand ECHO machine, Aplio 300 3MHz transducer and in accordance with the American echocardiography Society guidelines¹⁹. Three different measurements were taken at rest and the average recorded as the mean TRV, where values ≥ 2.5 m/s were classified as having elevated TRV and those < 2.5 m/s as normal. A value of 2.5m/s-2.9m/s was suggestive of mild rise in PASP and values of 3.0m/s or more regarded as moderate to severe rise.

Free plasma haemoglobin was determined using the solid phase sandwich ELISA technique. Optimization was pre-determined prior to running the test sample. The normal reference range of free plasma Hb in non-sickle cell patients is 10 - 40 μ g/ml²⁰. Free plasma haemoglobin values above 40 μ g/ml was regarded as abnormal and suggestive of ongoing intravascular haemolysis.

Data Analysis and Ethical consideration

All information from the questionnaire were captured and cleaned and later exported into Statistical Package for Social Sciences (SPSS) version 22 for data analysis. The Laboratory parameters, TRV and spirometry measurements (FVC and FEV₁) were summarized by means and standard deviations. Spearman's correlation was used to establish association between spirometry, laboratory and doppler echocardiogram findings. Linear regression and multivariate analysis were used to examine significant factors influencing TRV outcomes among the sickle cell patients.

Ethical Approval was obtained from the Ethical

and Protocol Review Committee of the College of Health science, University of Ghana. (Protocol identification number- CHS-Et/M.10-P3.9/2013-2014). Clearance was also received from the Head of the GICG where the review was conducted.

Results

Demographic and Clinical Data

The mean age for the SCA subjects was 33.6 \pm 11.1 years with a range of 18-63 years and majority (60.5%) were females. The mean height and weight were 164.3cm and 59.0kg respectively, with a mean BMI of 21.8kg/m² (Table 1). Clinical history showed that 6(7.9%) of the SCA subjects had hypertension, 1(1.3%) had diabetes and 1(1.3%) had chronic kidney disease (Table 2). Fifty-three (69.7%) of the participants had a history of previous blood transfusion and 28 (36.8%) had a history of chronic leg ulcer (Table 2). Nine (25.7%) out of the 30 male participants had a previous history of priapism, mostly during the adolescent period. Sixty-three (82.9%) had between 0 – 2 and 13 (17.1%) had 3 or more episodes of sickle cell crisis in the past year, mostly reported as bone pain (Table 2).

Laboratory Results

From Table 1, the mean steady state haemoglobin (Hb) was 8.06 \pm 1.59g/dl with a range of 4.9 – 11.2 g/dl, a mean MCV of 83.3 \pm 11.6fl and MCH of 28.5 \pm 4.3 pg respectively. The mean total white cell count (WBC) was elevated 10.1 \pm 3.6 $\times 10^9$ /l with a range of 3.4 – 26 $\times 10^9$ /l and that of the total platelet count was 341.4 \pm 109.2 $\times 10^9$, with a wide range of 143-873 $\times 10^9$ /l. Mean blood urea was, 3.5 \pm 1.6 mmols/l and that of serum creatinine was 55.6 \pm 20.0 mmols/l. The mean value of free plasma haemoglobin was 105.7 \pm 53.4 μ g/ml with a range of 8.2- 471.1 μ g/ml. Sixty-six (90.4%) of the SCA patients had intravascular haemolysis (free plasma Hb > 40 μ g/ml).

Echocardiogram and TRV

Approximately a third of the SCA participants, 25 (32.9%) had elevated TRV (≥ 2.5 m/s) with 19(76%) of them having mild elevation in TRV (2.5m/s - 2.9m/s) and 6(24.0%) with moderate-severe elevation (≥ 3.0 m/s). There was no significant difference between participants with elevated TRV and those with normal

Table 1: Descriptive summary of Anthropometric, Spirometry and laboratory measurements of SCA participants

Anthropometry results	Mean	Measured range	Std. Deviation
Weight (kg)	59.0	38.0-117.5	13.2
Height (cm)	164.3	135.0-182.5	8.2
BMI (kg/m ²)	21.8	15.8-40.2	4.2
Age (years)	33.8	18.0-63.0	11.1
Spirometry results			
FVC % Predicted	72.6		22.2
FEV1 % Predicted	65.3		20.6
% Ratio (FEV1/FVC)	75.6		9.6
Laboratory results			
Haemoglobin (g/dl)	8.06	4.9- 11.2	1.6
Haematocrit (%)	24.86	14.2- 83.0	8.6
MCV (fl)	83.27	78.0-105.0	11.6
MCH (pg)	28.53	16.3-36.5	4.3
Total WBC (10 ⁹ /l)	10.07	3.4-26.0	3.6
Platelet Count (10 ⁹ /l)	341.36	142.0-873.0	109.2
Free Plasma Hb (ug/ml)	105.70	8.2-471.1	53.4
Urea (mmol/l)	3.48	1.6-10.7	1.6
Creatinine (mmol/l)	55.85	21.0-118.0	20.0

Table 2: Clinical History of the SCA subjects

Clinical Parameter	Frequency (n)	Percent (%)
Hypertension	6	7.9
Diabetes	1	1.3
CKD	1	1.3
History of Leg Ulcer (n=76)	28	36.2
History of Blood Transfusion (n=76)	53	69.7
History of Priapism (n=30)	9	25.7
Frequency of Sickle Cell Crises		
1	26	34.2
2	11	14.5
3	6	7.9
>3	7	9.2
None	26	34.2
<i>Total</i>	<i>76</i>	<i>100</i>

Table 3: Comparisons of means of laboratory results with normal and elevated TRV

TRV (m/s)		Hb (g/dl)	Hct	MCV	MCH	Total WBC (x10 ⁹ /l)	Platelet Count (x10 ⁹ /l)	Plasma Haemoglobin	Urea (mmol/l)	Creatinine (mmol/l)
Normal	<i>Mean</i>	8.6	25.7	83.9	28.4	10.0	341.8	113.0	3.2	52.8
	<i>Std.</i>	1.3	4.2	8.3	3.9	3.3	115.0	56.1	1.0	14.0
Elevated	<i>Mean</i>	6.9	23.0	82.0	28.8	10.3	362.6	117.3	4.1	61.9
	<i>Std.</i>	1.6	13.8	16.6	5.1	4.3	144.8	102.9	2.3	27.7
P-value		0.001*	0.194	0.503	0.682	0.762	0.506	0.820	0.030*	0.088

TRV with regards to age, BMI and frequency of sickling crisis in a year ($p > 0.05$). There was however a significant association between elevated TRV and history of chronic/recurrent leg ulcers ($p=0.043$). The mean haemoglobin level was significantly different between those with elevated TRV and those without (6.9g/dl and 8.6g/dl respectively, $p < 0.001$), (Table 3). Further analysis showed that among participants with elevated TRV, those with values $\geq 3.0\text{m/s}$ had a significantly lower mean Hb of 5.8g/dl compared to 7.3g/dl in those with values between 2.5 – 2.9m/s ($p=0.023$). A significant inverse correlation was also observed between steady state Hb and TRV ($R= -0.456$, $p=0.001$, Figure 1) as well as with free plasma Hb ($R = -0.104$, $p = 0.039$). The positive correlation between TRV and free plasma Hb noted was however not statistically significant ($R=0.075$, $p=0.534$). Blood urea was significantly higher in the group with elevated TRV than in the normal TRV group ($p=0.030$) but there were no significant differences observed between the two groups with regards to total WBC count, platelet count, free plasma hemoglobin and creatinine level, $p > 0.005$ (Table 3).

Spirometry

With regards to lung function, there was a significant difference in the mean oxygen saturation of participants with elevated TRV and those without (92.0% and 94.0% respectively, $p = < 0.001$). Spirometry measurements of participants showed a mean FVC% predicted of 72.6 and a mean FEV₁% predicted of 65.3 with a mean FEV₁/FVC ratio of 75.6. There were no significant differences in the FEV₁ and FVC percentage predicted values of participants with elevated TRV and those without, $p>0.05$.

Discussion

Pulmonary hypertension (PH) complicated by right heart failure has emerged as an independent risk factor for morbidity and mortality among SCD patients, especially in those with homozygous disease. Assessment of TRV via trans-thoracic echocardiography has been shown to predict the presence of PH in SCD patients and has prognostic significance. Elevations in TRV $\geq 2.5\text{m/s}$ has been found to be associated with increased risk of mortality with a reported prevalence of about 25-30% among SCA patients^{6,7,8}. Moderate to severe elevation in TRV ($\geq 3.0\text{m/s}$) is associated with a

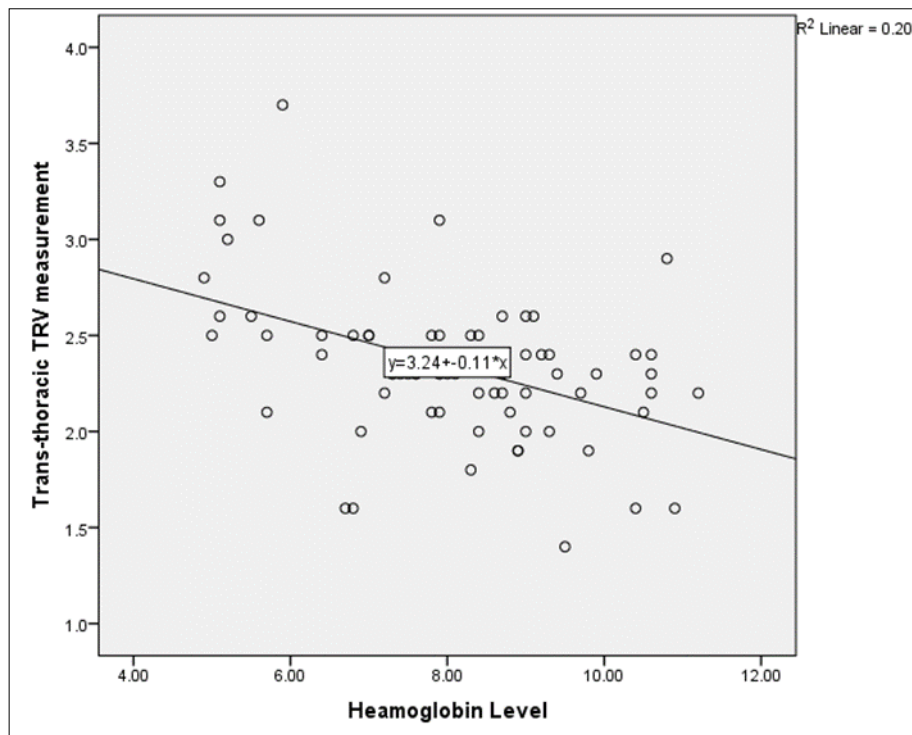


Fig 1: Correlation Between TRV and Haemoglobin

higher risk of mortality and an increased probability of definite diagnosis of pulmonary hypertension on RHC ^{7,12,21}. In the current study, 25(32.9%) had elevated TRV, $\geq 2.5\text{m/s}$. Among these, the majority(76%) had mild TRV elevation of between $2.5\text{m/s} - 2.9\text{m/s}$. Our prevalence is similar to the 32% reported by Gladwin et al, 2004, in which the mean age of the SCA participants (36 ± 12 years) is comparable to that of our cohort. A study by Aliyu et al found a prevalence of 25% among a cohort of younger Nigerian SCA patients with mean age 22 ± 8 years ¹¹. There is some suggestion of higher TRV values with increasing age of SCA patients and this could have accounted for the lower prevalence in the Nigerian study ²². Our study however, showed a positive but non-significant correlation between age and TRV measurements ($R=0.147$, $p=0.205$). The presence of elevated TRV in about a third of the participants revealed that PH as a complication of SCA may not be a rare occurrence among our SCA cohort, though this has not been previously studied. This result gives the realization that as recommended by the ATS adhoc committee on pulmonary hypertension, our SCA patients should also be periodically screened for early detection of PH ¹⁶.

The current study showed a low mean steady state haemoglobin, raised total WBC count, among the SCA subjects. These results are consistent with the common haematological profile of patients with SCA ²⁰. The baseline steady-state haemoglobin in sickle cell patients has been shown to be within the range of $6 - 9\text{g/dl}$ and this study similarly reported a mean of $8.06 \pm 1.59\text{g/dl}$ which was largely normocytic, normochromic ^{20,23}. Few participants had microcytic hypochromic anaemia which may have been due to coexisting iron deficiency anaemia; however iron status of the participants was not assessed. The proportion of participants with intravascular haemolysis was very high (90.41%) and this was not surprising as patients with SCA are known to have more severe chronic haemolysis ²⁰. Our analysis further showed a significant negative correlation between free plasma Hb (a marker of intravascular haemolysis) and steady-state Hb level ($R = - 0.104$, $P=0.039$). This suggests that intravascular haemolysis, may have been the major cause of the low steady state Hb observed.

Previous studies have shown that markers of intravascular haemolysis such as lactate dehydrogenase (LDH) and free plasma Hb, are significantly associated with vascular complications of sickle cell disease including PH, strokes, priapism, leg ulcers and renal failure ^{5,15,24}. There are also reported associations between the presence of PH, raised levels of free plasma Hb and Nitric oxide (NO) consumption within the vascular endothelium ²⁵⁻²⁷. These studies have proposed that endothelial NO, which maintains vascular integrity and vasodilatory function, are depleted by the release of toxic products such as free plasma Hb and arginase-1 from lysed red cells. Therefore, chronic intravascular haemolysis as occurs in sickle cell patients, leads to the depletion of haptoglobin and haemopexin, which are natural scavengers of free plasma Hb ²⁸. The blood level of free plasma Hb subsequently rises appreciably and exerts its effect by mopping up considerable amounts of Nitric Oxide (NO) from within the vascular endothelial cells. In addition, arginase 1, breaks down arginine, an important substrate for intracellular NO production. Both processes concurrently result in endothelial NO deficiency and the long-term effect is the development of sickle cell disease associated vasculopathy, a precursor to developing PH ^{25,26,29}.

The current study similarly found a significant inverse correlation between the steady state Hb and TRV as well as between steady state Hb and free plasma Hb ^{6, 30}. There was however no significant inverse correlation between TRV and free plasma Hb. This finding questions the proposed direct role of free plasma Hb induced NO deficiency in the aetiology of PH among SCA patients. In support of this, a study by Machado et al, found no evidence of reduction in the mean pulmonary artery systolic pressure or TRV measurements when SCA patients with RHC confirmed PH were treated with 5' phosphodiesterase inhibitors, a drug that increases bioavailability of NO within endothelial cells ³¹. We therefore suggest that the additive effect from other products of chronic haemolysis released from the lysed red cells, including endothelial adhesion factors, procoagulant factors, phosphatidyl serine, could be contributing to the pulmonary vascular endothelial damage, hence the absence of a direct association between TRV and free plasma haemoglobin

as observed in other studies ^{32,33}.

The effect of chronic hypoxia on the integrity of pulmonary vasculature has been shown to be an important cause of PH in SCA ³⁴. Some studies have shown that chronic anaemia in SCA is associated with chronic up-regulation of the hypoxic response through activation of hypoxia inducible factor (HIF- α) with attendant damage to the pulmonary vascular endothelium ³⁵. The strength of association between low steady state hb and TRV is emphasized by the evidence that chronic scheduled transfusions reduce most complications of SCD such as strokes, acute chest syndrome (ACS) and vaso-occlusive crisis (VOC) ³⁶. These benefits are achieved through reduction in the burden of sickled RBCs and the rate of haemolysis. Lezcano et al, 2006, also showed that chronic transfusions reduce free plasma Hb with subsequent lowering of TRV among SCD patients studied ³⁷. These findings together with that from the current study, show that improving the most significant risk factor, low steady-state haemoglobin, is key in preventing/reducing the occurrence of vascular complications of SCA patients such as PH.

Assessing the association with other vascular complications in SCA, the current study found a significant relationship between TRV and chronic/recurrent leg ulcers ($p = 0.043$) but not with priapism ($p=0.253$). Although we also found a significant association between serum urea and TRV, which compares with findings in previous studies, the former is not a measure of kidney function and measurement of urine microalbumin would have been a better marker of early renal involvement ^{6,11,38,39}.

Frequency of sickling crisis, which is considered a marker of disease severity among SCA patients was not statistically different between the groups with elevated TRV and those without ³⁹. The current study showed significantly lower mean oxygen saturation in patients with elevated TRV compared to those with normal values (92.0% and 94.0% respectively, $p < 0.001$). As similarly observed in previous studies, this could reflect the role of hypoxia in the development of PH ^{6,39}.

In conclusion, steady-state Hb is a significant

risk factor for elevated TRV in SCA, with increased risk when steady-state Hb is 6.9g/dl or less. In view of this finding, we recommend that steady-state Hb could be used to selectively identify SCA patients who need screening for PH, particularly in low resource settings. Also, measures to reduce or prevent hyperhaemolysis in SCA patients such as hydroxyurea and chronic scheduled transfusions should be considered in our setting, although the latter may not be immediately feasible in low income countries.

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Competing Interest

The authors have no competing interests.

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