Am J Blood Res 2021;11(2):199-205
www.AJBlood.us /ISSN:2160-1992/AJBR0132753

Original Article

Association between pulmonary function and cardiac enzymes in sickle cell disease

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Received March 3, 2021; Accepted April 14, 2021; Epub April 15, 2021; Published April 30, 2021

Abstract: Background: There is scarcity of data on association between lung function and cardiac markers in patients with sickle cell disease (SCD). Meanwhile, SCD affects multi-organs in any one population. There seem to be an association between reduced pulmonary function with cardiac dysfunction. The current study examined the association between pulomanry function with cardiac markers in patients with SCD. Methodology: This was a cross-sectional study with cases and controls. The cases (n=117) were made up of patients with SCD. The control subjects (n=58) were voluntary blood donors without SCD. The cellulose acetate electrophoresis was used to determine the genotypes of the study subjects. Blood samples were collected from all the study subjects for full blood count and measurement of cardiac enzymes. The cardiac enzymes measured were lactate dehydrogenase (LDH) and creatine kinase-myocardial band (CK-MB). Lung function test, using the vitalograph was done on all the study subjects. The Global Lung Initiative criteria were used to categorize lung disease as obstruction, restriction, mixed obstruction/restriction and normal. Results: The prevalence of elevated CK-MB and LDH among the SCD patients was 76.92% and 9.40% respectively, higher than the non-SCD controls (51.72% and 0% for elevated CK-MB and LDH respectively). Of all the impaired lung function, lung restriction was prevalent in all the study groups (30.77% and 15.52% for SCD patients and non-SCD controls respectively). In the fully adjusted model, reduced FEV1 was associated with nearly 3.5-fold higher odds of elevated CK-MB (odds ratio 3.35, 95% CI 1.26-8.90, *p*-value 0.015) in individuals with SCD. Conclusion: Reduced FEV_1 which reflects airflow impairments are associated with CK-MB elevations in patients with SCD, suggesting a possible damage to the cardiomyocytes.

Keywords: Sickle cell disease, lung function, myocardial infarction, cardiac enzymes

Introduction

Sickle cell disease (SCD) is a genetic disease that is associated with acute illness and affects multiple organ systems in anyone population [1, 2]. Although the incidence of SCD varies by state, race, and ethnicity, the disease is prevalent in sub-Saharan Africa. About 50-90% of children with sickle cell anaemia (SCA) die by age 5 years in sub-Saharan Africa [3]. In Ghana, about 2% of all births have been reported to have SCD [4]. The associated mortality and morbidity rates thus, cannot be overemphasized in developing countries such as Ghana [5].

Due to the polymerization of the HbS, free flow of blood through micro and macro vessels are impaired, contributing to vaso-occlusive crises and chronic haemolysis; which are the hallmarks of the disease, as well as endothelial dysfunction [6, 7]. The SCD affects the normal function of multiple organs including the lungs and the heart. Cardiopulmonary abnormalities are the most common causes of death in adults with SCD [8]. Advances in the management of SCD patients have contributed to the increased survival of patients. This has led to an increase in the occurrence of chronic organ damages [9, 10]. The associated chronic anaemia in patients with SCD results in cardiac chamber dilation, as

well as a compensatory increase in left ventricular mass, which leads to left ventricular diastolic dysfunction [11]. The frequent episodes of vaso-occlusive crises have also been reported to cause ischemia and tissue fibrosis in patients with SCD [12].

A potential modifiable risk factor for cardiac dysfunction in SCD is the role of pulmonary dysfunction [13, 14]. In the general population, forced expiratory volume expired in one second (FEV_1) percentage predicted is a known robust predictor of sudden cardiac death [15, 16].

Chronic lung disease has been reported to be associated with recurrent episodes of acute chest syndrome in SCD patients, which may be caused by factors including pulmonary infarction [17, 18]. In a previous study, a higher frequency of restrictive abnormalities was observed in patients with a history of acute chest syndrome as well as patients with increased left ventricle size, suggesting that these two systems (pulmonary and cardiovascular) may be related [14]. Reduced FEV_1 percentage predicted has also been associated with an increased risk of cardiovascular outcomes in young adults [19].

In Ghana, although some studies have been conducted to determine organ involvement in patients with SCD, these studies were done singly. Meanwhile, other studies have determined an association between restrictive lungs and cardiac abnormalities [14, 20]. No previous study has determined the association of lung function and cardiovascular abnormalities in Ghana. It is, therefore, important to understand the pathophysiology of these two organ systems in SCD, and determine whether poor pulmonary function in SCD, assessed by reduction in FEV_1 is associated with elevations in cardiac enzymes.

Methodology

Study design, subject recruitment, and data collection

This was a cross-sectional study with cases and controls, and was conducted at the center for clinical genetics of the Korle-Bu Teaching Hospital between the periods of January-March, 2020. The cases consisted of adult patients (>18 years) with established diagnosis of SCD (with Haemoglobin SS and SC), based on cellulose acetate hemoglobin electrophoresis at alkaline pH. The patients with SCD were in the steady-state, defined as a period of no blood transfusion with no episodes of VOC, infection, stroke, priapism, acute chest syndrome for at least 3 months prior to sample collection [21]. Patients with genotype HbSS and HbSC confirmed with alkaline electrophoresis with cellulose acetate membrane, who are at the steady state were included in the study. Exclusions are patients with SCD and co-morbid chronic conditions including seizure disorders, and history or clinical signs and symptoms of HIV infection. Data on anthropometry were collected from all the study subjects after they gave consent to partake in the study. Venous blood was drawn from all the study subjects into EDTA and gel separator tubes. Full blood count was done within two hours of sample collection. The blood specimens were allowed to clot at room temperature for 30 min and sera were obtained after centrifugation at 1000× *g* for 10 min. The sera were kept in Eppendorf tubes and stored at -80°C prior to analyses.

Laboratory analysis

The sera were used for determination of cardiac enzymes including CK-MB, and LDH using the BS-200 Chemistry Analyzer (Mindray, Shenzhen, China), following the manufacturer's protocol. The reference ranges were 0.0-16.0 U/L and 313.0-618.0 U/L for CK-MB and LDH respectively. Elevated CK-MB and LDH was defined as serum CK-MB and serum LDH level above 16.0 U/L and 618.0 U/L respectively.

Spirometry

Lung function test was done on all the study subjects using the VitalographAlpha model 6000 according to American Thoracic Society/European Respiratory Society guidelines (ATS). Measured and calculated spirometric indices from the FVC maneuver included FEV_1, FVC, and the ratio of FEV_1 to FVC (FEV_1/FVC). The predicted values of the FEV_1, FVC, and the FEV_1/FVC ratio were determined for each participant based on their age, gender, height, and ethnic group using the Global Lung Initiative 2012 equations [22]. The values of FEV_1/FVC and FVC was used to categorize pulmonary function patterns as normal, obstructive, rest-

Table 1. Anthropometry and clinical characteristics of the study subjects

Parameter	Non-SCD (n=58)	Patients with SCD (n=117)	*P*-value
Age	31.09±13.24	28.74±11.48	0.228
Male n (%)	21 (36.21)	46 (39.32)	0.691
Female n (%)	37 (63.79)	71 (60.68)	
BMI, kg/m^2	27.66±6.70	21.72±4.61	<0.001
DBP, mmHg	75.19±11.90	71.38±10.53	0.033
SBP, mmHg	121.55±15.51	115.27±14.734	0.009
HR, bpm	78.48±11.27	82.44±12.19	0.040
Hb, g/dl	12.60±1.58	9.11±1.82	<0.001
WBC, (×10^9/L)	6.13±2.16	9.87±4.08	<0.001
PLT, (×10^9/L)	258.71±75.66	397.97±163.09	<0.001

Data is presented as mean ± standard deviation or frequencies. BMI: Body mass index; DBP: Diastolic blood pressure; SBP: Systolic blood pressure; HR: Heart rate; WBC: White blood cell; Hb: Haemoglobin; PLT: Platelet. HbAA: Apparently healthy individuals; HbSC: Sickle cell patients with haemoglobin SC genotype; HbSS: Sickle cell patients with haemoglobin SS genotype; HbAA: Apparently healthy individuals; HbSC: Sickle cell patients with haemoglobin SC genotype; HbSS: Sickle cell patients with haemoglobin SS genotype.

rictive or mixed obstructive and restrictive based on American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines according to a modified algorithm based on Pellegrino [23]. Based on the ATS guidelines, we defined reduced FEV_1 as FEV_1% predicted <70.

Data analyses

The data were entered in to SPSS version-22 software (IBM SPSS Statistics, Chicago, IL, USA). Frequency tables were generated for nominal and ordinal variables. The results are expressed as mean plus or minus standard deviation (mean ± SD) or median (interquartile range). The unpaired Student's t-test was used to compare the different parameters between the two subgroups; Non-SCD and SCD patients. A logistic regression model was developed to determine association of reduced pulmonary function (FEV_1 percentage predicted) with CK-MB elevations in the study subjects. Statistical significance was considered at P<0.05.

Ethical statement

Ethical approval for the study was sought from the Korle-Bu Teaching Hospital Scientific and Technical Committee/Institutional review board. The protocol identification number given was STC/IRB/00045/2019. Blood samples and demographic data were obtained from study participants following their consent to partake in the study.

Results

Anthropometric and clinical characteristics of the study subjects

The study groups (Non-SCD patients versus patients with SCD) were similar with respect to age (P=0.228). Gender distribution was not significantly different (P=0.691). Patients with SCD had significantly higher WBC (P≤0.001) and PLT counts (P≤0.001). The Hb level was however lower in patients with SCD compared to the non-SCD controls (P<0.001). Additionally, both DBP (P=0.033) and SBP (P=0.009) were significantly higher in the non-SCD controls (**Table 1**).

Measures of cardiac and pulmonary function

The means for FEV_1% predicted and FVC% predicted were significantly higher in the non-SCD group (P=0.002 and 0.003 respectively). Most of the patients with SCD were noted to have lung restriction. Mixed obstruction/restriction pattern was relatively low, occurring in 6.9% of non-SCD controls and 10.26% of patients with SCD. The prevalence of airway obstruction was comparable in the study groups (13.79% versus 13.67% for non-SCD controls and patients with SCD respectively). Compared to the non-SCD controls, most of the patients with SCD had elevated CK-MB (76.92% versus 51.72) and LDH (9.40% versus 0%) (**Table 2**).

Association between FEV_1 and elevated cardiac enzymes (CK-MB and LDH) in the study subjects

In the fully adjusted model, reduced FEV_1 was associated with nearly 3.5-fold higher odds of elevated CK-MB (odds ratio 3.35, 95% CI 1.26-8.90, *p*-value 0.015) in individuals with SCD. There were no associations between reduced FEV_1 and elevated CK-MB in the non-SCD controls. In the analysis of the association between reduced FEV_1 and LDH, there were no

Table 2. Measures of cardiac and pulmonary function

Parameter	Non-SCD (n=58)	Patients with SCD (n=117)	*P*-value
FEV_1% predicted	87.35±21.31	76.34±22.58	0.002
FVC% predicted	92.09±22.08	81.58±21.32	0.003
FEV_1/FVC ratio	0.81±0.09	0.80±0.09	0.555
Pulmonary Pattern			
Normal, n (%)	37 (63.79)	53 (45.30)	0.087
Airway Obstruction, n (%)	8 (13.79)	16 (13.67)	
Lung Restriction, n (%)	9 (15.52)	36 (30.77)	
Mixed obstruction/restriction, n (%)	4 (6.90)	12 (10.26)	
FEV_1% predicted <70, n (%)	49 (84.48)	70 (59.83)	<0.001
CK-MB, U/L	17.00 (24.20)	37.40 (61.20)	<0.001
Elevated CK-MB, n (%)	30 (51.72)	90 (76.92)	<0.001
LDH, U/L	70.75 (32.88)	312.20 (268.15)	<0.001
Elevated LDH, n (%)	0 (0)	11 (9.40)	0.016

Data is presented as mean ± standard deviation or median (interquartile range). AST: Aspartate transaminase; LDH: Lactate dehydrogenase; CK-MB: creatine kinase-myocardial band. HbAA: Apparently healthy individuals; HbSC: Sickle cell patients with haemoglobin SC genotype; HbSS: Sickle cell patients with haemoglobin SS genotype.

significant associations in the study groups (**Table 3**).

Discussion

In our study, pulmonary function (reduced FEV_1 percentage predicted) was associated with elevated CK-MB in a fully adjusted model. Elevated cardiac enzymes were mostly observed in patients with SCD. It was further noted that, lung restriction was predominant among the SCD patients.

The observation of CK-MB elevations in the SCD patients suggest a possible subtle damage to the cardiomyocytes which may lead to cardiovascular abnormalities such as acute myocardial injury. In a previous case report in a sickle cell patient, it was noted that, CK-MB level was elevated which conformed to the electrocardiogram (ECG) changes for diagnosing myocardial injury [24].

Another case report revealed abnormally high CK-MB levels in a patient with SCD suggesting myocardial infarction [25]. The elevated CK-MB in the serum may be due to at least in part, oxidative stress which is usually observed in patients with SCD. Majority of the SCD patients had elevated CK-MB levels, which is consistent with other previous studies [24, 25].

Abnormalities of spirometry outcomes such as reduced FEV_1 and FVC percentage predicted have been associated with cardiovascular events in a recent study [26]. In this current study, it was observed that, FEV_1 percentage predicted correlated significantly with CK-MB in the SCD patients, suggesting the contribution of lung function in cardiac abnormalities. Reduced FEV_1 percentage predicted was associated with an increased risk of cardiovascular outcomes in young adults [20]. In another study, a decline in FEV_1/FVC ratio was also associated with smaller atrial internal dimension as well as lower cardiac output [19]. Reduction in FEV_1 percentage predicted was also reported as a predictor of heart failure in a previous study [27]. Therefore, pulmonary function may serve as a good indicator of cardiac dysfunction in patients with SCD. The importance of routine assessment of pulmonary function in patients with SCD cannot be overemphasized.

In this study, FEV_1 percentage predictor was noted as a predictor of elevated CK-MB in the SCD patients. Reduced FEV_1 which reflects airflow obstruction has also been previously associated with cardiac dysfunction and increased risk of incident heart failure in older men [28]. In the study by Wannamethee *et al*. [28], FEV_1 and FVC were inversely associated with cardiac enzymes such as N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac troponins. The results from this study suggest that, airway obstruction which leads to decrease flow rates and lung hyperinflation may

Table 3. Association between FEV_1 and elevated cardiac enzymes (CK-MB and LDH) in the study subjects

	Non-SCD			Patients with SCD		
	OR (95% CI), *P*-value			OR (95% CI), *P*-value		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
CK-MB (>16 IU/L)	0.83 (0.20 to 3.48), 0.803	0.91 (0.21 to 4.03), 0.904	1.04 (0.22 to 4.95), 0.965	2.77 (1.15 to 6.69), 0.024	2.61 (1.06 to 6.42), 0.037	3.35 (1.26 to 8.90), 0.015
LDH	-	-	-	0.53 (0.13 to 2.10), 0.366	0.56 (0.13 to 2.33), 0.425	0.48 (0.11 to 2.08), 0.328

Model 1 was unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, BMI, Hb concentration and systolic BP. BMI: Body Mass Index; Hb: Haemoglobin; BP: Blood Pressure. None of the non-SCD individuals had elevated LDH.

have contributed to a decreased cardiac function in the patients, causing the release of CK-MB from the cardiomyocytes into the serum. The lung function decline may also contribute to an increase in cardiac pumping function to compensate for oxygen delivery to the body tissues. This compensatory mechanism may in turn result in cardiac and vascular overloads, as well as cardiovascular events [29]. Additionally, hypoxia/hypoxemia and poor coronary perfusion may explain mechanisms linking low FEV_1 and possible cardiac dysfunction in SCD.

It would have been very interesting to determine association of reduced FEV_1 and FVC with other specific cardiac biomarkers such as the cardiac troponins and NT-proBNP. These cardiac biomarkers were not measured in the study, and we duly acknowledge it as a limitation of the study. This presents the first study in SCD to determine an association between pulmonary function and cardiac enzyme. The positive association of reduced FEV_1 and elevated CK-MB in the SCD patients suggest that lung dysfunction may lead to some cardiovascular events. The mechanism linking impaired lung function and cardiovascular disease needs to be elucidated. Additionally, a better understanding of the association between pulmonary function and cardiovascular outcomes could be achieved with a larger sample.

Conclusion

Reduced pulmonary function (FEV_1 percentage predicted) which reflects airflow impairments is associated with CK-MB elevations in patients with SCD, suggesting a possible damage to the cardiomyocytes. In patients with SCD, interventions aimed at addressing reduction in pulmonary function may help reduce the risk of cardiovascular abnormalities.

Disclosure of conflict of interest

None.

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