Lung Function and Inflammation in Adult Sickle Cell Disease in a West Indian Sickle Cell Cohort

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Abstract

Background: The commonest cause of mortality in sickle cell disease (SCD) is an acute condition called the acute chest syndrome. Little is known of the long term effects of this condition or of its effect on lung function.

Objective: To determine lung function in sickle cell disease (SCD) adults and its relation to acute chest syndrome, baseline haemoglobin and systemic inflammation as estimated by serum C-reactive protein.

Methods: The lung function in sickle cell disease (SCD) outpatients was compared to that in asymptomatic age and sex matched controls. Stable haemoglobin and serum C-reactive protein (CRP) were measured.

Results: 74 controls of mean age 31 years and 154 sickle cell disease (SCD) outpatients with mean age 31 with 44% males were recruited. Controls and sickle cell disease (SCD) patients did not differ in age or sex. 18% sickle cell disease (SCD) patients had at least one episode of acute chest syndrome and 7% had two or more episodes. Forced vital capacity was lower in sickle cell disease (SCD) patients than controls and 47% of sickle cell disease (SCD) patients had a restrictive ventilatory defect. A further 19% had obstruction but the presence of this abnormality was not related to smoking habit. Patients with a history of two or more acute chest syndrome episodes were more likely to have poor lung function. Haemoglobin was positively correlated with forced vital capacity and inversely correlated C-reactive protein. Multivariate analysis showed that haemoglobin in sickle cell disease (SCD) patients was lower if patients also had a low forced vital capacity, high serum C-reactive protein, low body mass index, older age or male gender.

Conclusions: Abnormal lung function is common in sickle cell disease (SCD) adults. In sickle cell disease (SCD), adult's low haemoglobin is related to a restrictive ventilatory defect and systemic inflammation. Severity of stable disease in sickle cell patients is related to poor lung function and systemic inflammation.

Introduction

Sickle Cell Disease (SCD) is the most common inherited disorder affecting African and Caribbean populations [1]. Sickle Cell Disease (SCD) represents a spectrum of inherited haemoglobin disorders which include a number of genotypes namely homozygous sickle cell disease (haemoglobin SS disease), doubly heterozygous sickle haemoglobin C disease (haemoglobin SC disease) and the sickle ß-thalassemias [2]. These genotypes are associated with a wide range of phenotypes characterized by chronic haemolysis, tissue infarction, and painful episodes [3].

Acute Chest Syndrome (ACS) is a multifactorial process with the likely final common pathway being in situ microvascular thrombosis [4]. It is a life threatening condition and a frequent cause of death among sickle-cell patients being one of the more common causes of their morbidity and hospitalizations [5,6]. The prevalence of acute chest syndrome among sickle cell disease (SCD) patients in the Trinidadian population is currently unknown. A 6-month study conducted at the Port-of-Spain General Hospital, Trinidad and Tobago, showed that of 82 admissions for painful crises in sicklers, 13% were due to the acute chest syndrome [6]. It has also been shown that although acute chest syndrome is often a self-limiting illness, its effects on lung function may be quite significant, leading to life-threatening respiratory insufficiency and some have speculated that it may lead to development of chronic lung disease [7]. Anecdotal reports from our chest clinic suggested that our sickle cell patients may have a restrictive ventilatory defect.

Apart from lung function, blood markers have also been shown to be related to clinical episodes in patients with sickle cell disease.

Lower baseline haemoglobin levels are associated with an increased rate of painful episodes and morbidity in sickle cell disease [8]. Acute phase reactants such as C-reactive protein (CRP) have been found to be significantly elevated in sickle cell patients, particularly in those with homozygous disease [9].

We compared lung function in a group of sickle cell disease (SCD) patients and controls and hypothesized that our sickle cell outpatients have a restrictive ventilatory defect. We also explored relationships between the severity of the anaemia in sickle cell disease patients and lung function variables and serum CRP in this study.

Methodology

A patient was taken as having the acute chest syndrome (ACS), if this was stated as the medical diagnosis in the hospital discharge notes.

Sample population and inclusion and exclusion criteria

Sickle cell disease patients: A total of 361 sickle cell disease patients

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were registered at the Haematology Clinic, Port of Spain General Hospital. 154 patients (HbSS=143; HbSC=7; HbS beta-thal=4) were actually recruited. There was no difference in mean age or gender of sickle cell patients sampled and those not sampled. Twenty-seven of the 154 recruited patients had a history of at least one episode of acute chest syndrome. A history of acute chest syndrome was obtained by direct communication via a questionnaire and confirmed by examination of the patient file for a specific and documented diagnosis of the syndrome. Patients were recruited only if they did not have any existing pulmonary disorders [10], or any medical conditions that may have led to suboptimal results [11] or which may have been exacerbated by the testing procedure [12]. Of the total eligible population, eight patients refused to participate, six were recently deceased, four were excluded for medical reasons namely HIV, viral hepatitis (2 patients) and pregnancy, and 189 patients were unavailable or could not be contacted.

Healthy subjects: We recruited 74 ethnically matched healthy subjects (controls) with a smoking history of less than 5 pack years [13]. All controls had no medical symptoms or history of medical illness and also had normal lung function that is, FEV1 greater than 80% predicted for age and height and FEV1/FVC greater than 70%.

Ethical review

The study was approved by the institutional review boards of the University of the West Indies and Port of Spain General Hospital, Trinidad and Tobago. All subjects gave written informed consent.

Lung function

Spirometry measurements were performed with a hand-held turbine spirometer (MicroSpirometerCat No: MS01 Micromedical Limited P. O Box 6 Rochester, Kent, ME1 2AZ, UK), according to Global Initiative for Obstructive Lung Disease Criteria [14]. Post bronchodilator spirometry was taken for all sickle cell disease patients. Since by definition normal subjects had no history of disease or symptoms of illness, this was not a prerequisite for their spirometry measurements. To calculate the predicted value for lung function, we used the Third National Health and Nutrition Examination Survey (NHANES-III) Hankinson's reference equations for afro-Americans [15], according to age, sex, and height. An obstructive ventilatory defect was defined as an FEV1/FVC ratio less than 70% while a restrictive ventilatory defect was taken as an FVC% predicted for age and height of less than 80%.

Other measurements

Non-fasting venous blood samples were obtained from sickle cell disease patients for serum CRP analysis and plasma samples with ethylene diamine tetra acetic acid (EDTA) as anticoagulant used for haemoglobin determination. Serum CRP concentrations were measured using the Tina-Quant sCRP (Latex) high sensitive immunoturbidimetric assay on the Roche/Hitachi 912 Automatic Analyzer (Roche Diagnostics, GmbH, D-68298 Mannheim). The measuring range was 0.1-20 mg/L with a detection limit of 0.03 mg/L.

Statistical analysis

We assumed that controls would have a mean (SD) FEV1 of 100 (12%) predicted for age and height. At the 5% level of significance with a power of 95%, we estimated that the sample size required to detect an 8% difference in mean FEV1 in sickle cell disease subjects was 60 [16]. Data was analysed using SPSS (version 12 for Windows). Normally distributed data were summarised by mean (standard deviation, SD), skewed data by median (interquartile range, IQR) and categorical data by absolute number (percentage). CRP was normalized by log transformation.

Variable	Controls	SCD	p-value
N	74	154	
Age	30.7 (10.3)	33.8 (12.0)	0.053
Height, m	1.71 (0.093)	1.69 (0.09)	0.064
FEV1 (L)	3.13 (0.62)	2.34 (0.66)	<0.001
FEV1%	94.9 (11.6)	74.5 (13.3)	<0.001
FVC (L)	3.83 (0.79)	3.13 (0.93)	<0.001
FVC%	97.6 (12.1)	83.6 (16.3)	<0.001
FEV1/FVC%	82.0 (5.6)	75.5 (11.0)	<0.001

Abbreviations: SCD: Sickle Cell Disease; ACS: Acute Chest Syndrome; N: Number; M: Male; BMI: Body Mass Index

Table 1: Baseline data/confounding variables for 74 controls and 154 sickle cell disease patients. Data are shown as mean (SD) or IQR (25th centile, 75th centile) or number (%). There were N = 36 (48.6%) males amongst the controls and 67 (43.5%) sickle cell disease patients. BMI for sickle cell disease patients was 21.0 (4.2) kg per square metre.

Variable	Correlation w/ SCD	p-value
Age	0.123	0.099
Gender	-0.106	0.157
Height	-0.167	0.025
FEV1	-0.566	<0.001
FEV1%	-0.652	<0.001
FVC	-0.432	<0.001
FVC%	-0.466	<0.001
FEV1/FVC	-0.317	<0.001

Table 2: Spearman's Correlations amongst never-smokers between baseline variables and presence of sickle cell disease (65 controls and 115 sickle cell disease patients).

Normally distributed continuous variables were compared by t-test; otherwise Mann-Whitney U or Wilcoxon signed ranks test were used.

One-way ANOVA was used to examine the relationship of haemoglobin in sickle cell disease patients with lung function categorized as (A) normal lung function, (B) either restriction or obstruction but not both and (C) both obstruction and restriction. Backward linear regression with plasma haemoglobin as outcome variable was used to examine the relationship between baseline variables and haemoglobin as an estimator of severity of sickle cell anaemia. Gender and all variables with significant univariate relation to Hb were entered into the multivariate analysis. All data was analysed using SPSS version 12 (for Windows).

Results

Demographic and physiologic variables: all study participants

We recruited 154 sickle cell disease patients and 74 controls. CRP data was available for 138 and Hb data for 149 sickle cell disease patients. The baseline and demographic variables for controls and for sickle cell patients are shown in Table 1. 1.27 (18%) of the sickle cell disease patients had a history of acute chest syndrome (ACS). 17 (11%) sickle cell disease patients had one acute chest syndrome episode only, 5 (3.2%) patients had two episodes and 3 patients had three or more episodes. 9 (12%) of controls and 39 (25%) of the sickle cell disease patients sampled were smokers. The normal subjects did not differ from the sickle cell population in age and gender. Sickle cell patients had a mean (SD) height of 1.69 (0.88) m and weight of 60.0 (12.4) kg. In the sickle cell disease patients, blood results showed mean (SD) Hb was 8.97 (2.23) g/dl and median (IQR) CRP was 3.15 (1.43, 8.58) mg/L.
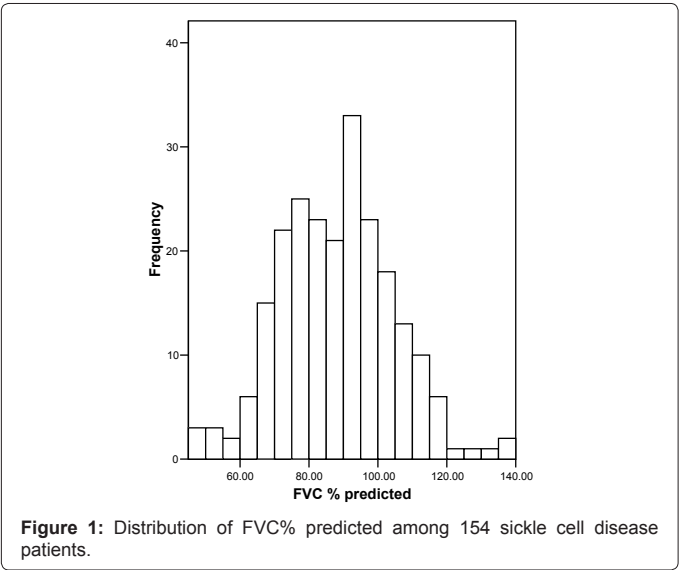
Lung function in controls vs. sickle cell disease

Lung function among controls and sickle cell disease patients is

Number (%)		Restriction**		Total
		No	Yes	
Obstruction*	No	52 (34)	60 (39)	112 (73)
	Yes	30 (19)	12 (8)	42 (27)
Total		82 (53)	72 (47)	154 (100)

* Obstruction was defined as FEV1/FVC less than 70%
** Restriction was defined as FVC less than 80% predicted for age and height

Table 3: The distribution of obstructive and restrictive ventilatory defects in 154 patients with sickle cell disease. The number of sickle cell disease patients (percentage of total) is shown.

Figure 1: Distribution of FVC% predicted among 154 sickle cell disease patients.

shown in Table 1. Lung function was higher in controls than in sickle cell disease patients in every parameter measured (Table 1) with controls having a median FEV1% of 94%. Median (range) FVC% in sickle cell disease patients was 82%.

Whether or not smokers were included in the analysis, sickle cell disease patients as a group still had significant inverse relationships with all lung function parameters when compared to the control group. Because smoking history can be a confounder in lung function analyses, we show the data for relationships with lung function in never-smokers amongst both the control and sickle cell disease samples in Table 2. The Table shows that the highest of the correlations between lung function in sickle cell disease vs. controls was -0.652 between FEV1% and presence of sickle cell disease. These values did not changed markedly when smokers were included in the analysis.

Lung function in sickle cell disease (SCD) patients

Lung function abnormalities in sickle cell disease (SCD), 52 sickle cell disease patients had normal lung function. Table 3 shows that 30 (19.5%) sickle cell disease patients had a FEV1/FVC ratio of less than 70% indicating obstruction. 12 (7.8%) had a mixed obstructive-restrictive ventilatory defect and 60 (39%) had a purely restrictive defect. 4 (5.4%) of the 74 controls had a restrictive defect. Figure 1 shows the distribution of FVC% in the sickle cell disease patients. 72 (46.8%) sickle cell disease patients were found to have a restrictive defect (including those with mixed defects).

Lung function and ACS, there was no statistically significant difference in the measures of lung function between sickle cell disease patients with a history of acute chest syndrome and those who never had an episode. However, patients with one or less acute chest syndrome

episodes had a FEV1% of 75.16 (13.25%) vs. 65.74 (10.89%) in those with two or more episodes (p=0.030). There was also a non-significant trend toward lower FVC%: one or less acute chest syndrome episodes had a FEV1% of 84.0 (16.35%) vs. 77.9 (15.3%), p=0.255.

Lung function and haemoglobin in SCD patients

Bivariate analyses showed that stable haemoglobin (Hb) was inversely correlated to age (r=-0.214, p=0.009), BMI (0.270, 0.001), abnormal (restrictive or obstructive) lung function (-0.286, p<0.001), presence of a restrictive ventilatory defect (-0.261, p=0.001), CRP (log) (-0.240, 0.005) and FVC% (0.286<0.001) but not other indices of lung function, history of smoking or height.

When sickle cell disease patients were categorized by lung function (Table 3) there was a trend (r=-0.269, p=0.001) in mean (SD) Hb amongst sickle cell disease patients grouped as follows: (Group A) normal lung function Hb=9.66 (2.25); (Group B) either obstruction or restriction but not both Hb=8.36 (2.03) and (Group C) both obstruction and restriction Hb=8.13(2.53). Application of one-way ANOVA confirmed the relationship and post hoc (LSD) testing showed that Groups B and C were both different from Group A but did not differ significantly from each other.

Multivariate linear regression with sickle cell patient plasma haemoglobin as outcome variable showed that regardless of age and gender there was a significant relationship between plasma haemoglobin and restrictive lung function as well as CRP (log) (Table 4). Figure 2 illustrates the relationship between plasma haemoglobin and FVC% with correlation r=0.286, p<0.001, in sickle cell disease patients.

Discussion

This is the first study of lung function and inflammation in sickle

Variable	B	p-value
Restrictive ventilatory defect	-0.689	0.049
Age	-0.036	0.014
BMI	0.179	<0.001
CRP (log)	-0.469	0.001
Male Gender	0.915	0.010

BMI = Body Mass Index
CRP (log) = Natural Logarithm of C reactive protein measured in mg/L

Table 4: Linear regression with haemoglobin as outcome variable in 154 sickle cell disease patients.

Figure 2: The relation between FVC% predicted and Hb in 149 sickle cell patients, r = 0.286, p < 0.001.
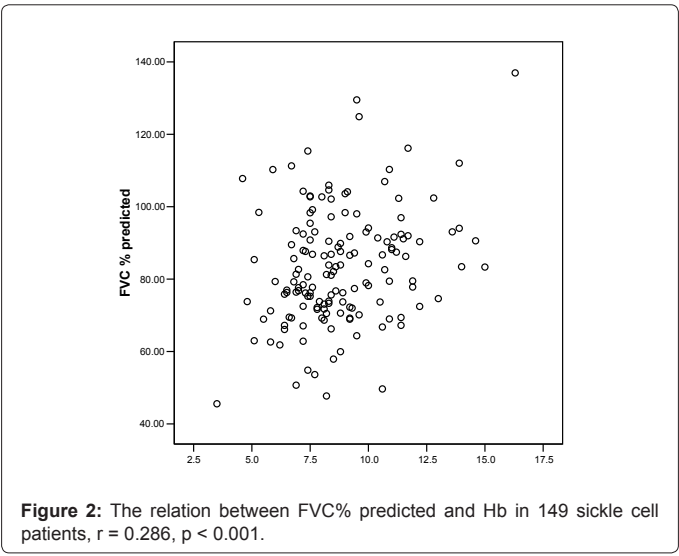
cell disease patients. In this study we found a prevalence of 18% with the acute chest syndrome (ACS) in our sickle cell disease patients. We found that about one-fifth of our sickle cell disease patients had an obstructive ventilatory defect and 39% had a restrictive-only ventilatory defect and so taken together 66% of sickle cell disease patients studied had abnormal lung function. We found that recurrent acute chest syndrome episodes were associated with a lower FEV1%. Plasma haemoglobin was lower in patients with a restrictive ventilatory defect and those with higher CRP.

We found a predominantly restrictive ventilatory defect in 47% of our sickle cell disease patients. This is less so, than was found in Jamaica (60%) [17]. In a study conducted at the Montreal Children's Hospital decreased lung volumes were thought to be the result of smaller thoraxes and a disparity between torso and limb growth among children with sickle cell disease [16]. Sylvester et al. [1] also found reduced height and lung volumes in children with sickle cell disease compared to controls and reported that this restrictive tendency increases with increasing age. Therefore, there is a tendency towards restriction among sickle cell disease patients from early childhood which progresses into adulthood and with which our results are consistent. In addition, unlike the Jamaican and USA studies, not all of our patients were homozygous for SS disease, the latter of which has been linked to disease severity [18].

Although the dominant pattern of abnormal lung function was restrictive, 19.5% of our sickle cell disease patients were found to have an obstructive defect and 7.8% had mixed disease. Vendramini et al. [17] reported a high prevalence of airway hyperresponsiveness and obstructive disease in adult patients with sickle cell disease when compared to controls, though obstruction was not seen in the Jamaican study [19]. Post-bronchodilator spirometry was used in our patients. Thus, we are unable to comment on any relationships with reversibility or airway hyperresponsiveness. However an in vitro study of sickle cell disease using the mouse model has found increased large and small airway resistance in the airways, if sickle cell disease mice and mechanism may underlie the spirometric lesions seen in our patients [20].

Our study is only the second study to report a relationship between frequent acute chest syndrome episodes and lung function in sickle cell disease in West Indian patients. The Jamaican study which reported this relationship above, found that 32.5% of sickle cell disease patients had two or more acute chest syndrome episodes as compared to 7% in our study [19]. Our patients of similar height, tended to have a greater weight and this may perhaps indicate a better nutritional status than their Jamaican counterparts. Nutritional status has been shown to ameliorate severity in sickle cell disease in one in vitro study [21].

We found that there is an inverse relationship between serum CRP and plasma haemoglobin in our sickle cell disease patients. The relation between systemic inflammation and sickle cell disease has previously been shown in children [22] and adolescents [23]. In several mouse models, inflammatory markers have been found in the sickle cell disease lung [24]. Importantly interleukin-6 (IL-6), a precursor of CRP, was elevated about two-fold in the sickle cell lung. Thus apart from a systemic stimulus to CRP formation in sickle cell disease there may also be a pulmonary stimulus as has been suggested in another model of chronic lung disease [25,26].

It has been shown that lower baseline haemoglobin is associated with an increased rate of painful episodes and morbidity in sickle cell disease patients [8], and that the percentage of haemoglobin F is a powerful predictor of clinical severity in these patients [27]. Our study showed that there is an association between lower steady-state haemoglobin

and the presence of a restrictive ventilatory defect. Moreover the lower the haemoglobin level, the higher the CRP, suggesting that chronic systemic inflammation is likely to play a role in the pathophysiology of the restrictive lung disease in these patients. These relationships support the hypothesis that the steady-state haemoglobin may be considered a surrogate marker of severity in the sickle cell disease in our population.

The inability to fully sample the entire patient population registered at the Haematology Clinic was a limitation to the study. Post-bronchodilator spirometry was used and this may have masked any reversible obstructive defect in the sample population. We did not perform postbronchodilator spirometry on our controls as they were asymptomatic and had no history of medical illness. If anything this would have caused us to under estimate the difference in lung function parameters between controls and sickle cell disease patients. The Hankinson prediction equations for Afro-Americans were used in this study to define normal ranges for lung function parameters as no reference ranges for a Trinidadian population are available [15]. We overcame this limitation by using a control group. Further we relied exclusively on hospital notes thus we may have excluded those patients who may have been treated in the community, if any. Finally, smoking data was obtained primarily by self-reporting and this may have led to an under-representation of the true number of smokers in the two sample populations.

In conclusion, this study confirms that in the Trinidadian population those with sickle cell disease tend to have lower lung function parameters than healthy controls consistent with a restrictive defect. In sickle cell patients low stable plasma haemoglobin is more likely in patients who have restricted lung function, who are older and who have more systemic inflammation and low BMI. Recently molecular markers rather than biophysical markers of severity have received most attention in sickle cell disease [28]. Throughout the last century, sickle cell disease has been studied at several physiologic levels (molecular, tissue, cellular and biochemical) little consensus has emerged on markers of disease severity [29,30]. Though our study was not designed to detect markers of severity in sickle cell disease, the relation of the easily measured plasma haemoglobin with FVC% and CRP lends support once again to the hypothesis that stable haemoglobin can be taken as a surrogate marker of severity in sickle cell disease.

Acknowledgements

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