

Pulmonary Function and Airway Hyperresponsiveness in Adults with Sickle Cell Disease

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Received: 3 November 2008 / Accepted: 16 February 2009
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Abstract *Study Objectives* Pulmonary involvement is a major cause of morbidity and mortality in patients with sickle cell disease (SCD). Although a high prevalence of airway hyperresponsiveness (AHR) has been reported, there are no studies demonstrating the relationship between AHR and acute chest syndrome (ACS) in adults with SCD. We investigated AHR prevalence, lung function abnormalities, and the relationships of these variables with ACS in SCD patients. *Method* Thirty-one adult patients without asthmatic symptoms were compared with 31 matched controls. Expiratory flow rates, lung volumes, carbon monoxide diffusion capacity (DLCO), and methacholine provocation test (MPT) results were assessed. *Results* Forced vital capacity (FVC), forced expiratory volume in one second, forced expiratory flow rate at 25% to 75% of FVC (FEF_{25%–75%}), peak expiratory flow rate, total lung capacity, and DLCO values were significantly lower in the patient group than in the controls. No significant difference in pulmonary function test results was found between patients with and without a history of ACS. Fifteen patients

with SCD (48%) and only 5 controls (16%) had AHR ($p = 0.007$). A significant correlation was found between the number of ACS episodes and MPT positivity ($r = 0.379$, $p = 0.035$). The FEF_{25%–75%} values were significantly lower in patients with positive MPT results than in patients with negative MPT results ($p = 0.027$). *Conclusion* The prevalence of AHR was high in adult patients with SCD. A significant correlation was found between AHR and recurrent ACS episodes. Anti-inflammatory controller agents can be used routinely to decrease pulmonary morbidity associated with SCD, even in the absence of asthmatic symptoms.

Keywords Sickle cell disease · Lung function · Airway hyperresponsiveness · Acute chest syndrome

Introduction

Pulmonary involvement is a cause of increased morbidity and a major determinant of survival in patients with sickle cell disease (SCD). Two main pulmonary complications have been defined: chronic sickle cell lung disease (CSLD) (which is initiated by recurrent vascular endothelial damage and results in chronic fibrosis) and acute chest syndrome (ACS) (which, when it recurs, is thought to be related to increased risk of CSLD) [1–3]. To date, different types of ventilatory defects such as restrictive lung disease, abnormal carbon monoxide diffusion capacity (DLCO), obstructive lung disease, hypoxemia, and pulmonary hypertension have been defined in SCD patients [2, 4–8]. The major abnormality in adult patients is restrictive defect [2, 7], whereas the most common defect in children with SCD is airway obstruction [5, 9].

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Airway hyperresponsiveness (AHR) is characterized by transient lower airway obstruction in response to different inhaled agents, cold air, or exercise. The increased prevalence of AHR in patients with SCD has been shown in previous studies [10–13]. However, the mechanism of increased AHR and its relationship with ACS have yet to be defined. The aims of this study were to investigate the lung function abnormalities in adult patients with SCD and compare them with persons in a healthy control group, to determine the prevalence of AHR, and to investigate the relationship between these variables and ACS episodes.

Materials and Methods

This study included patients with SCD followed regularly at the hematology clinic of the Baskent University Faculty of Medicine, Adana Teaching and Medical Research Center. The study protocol was approved by the Baskent University Ethical Committee, and written informed consent was obtained from all of the patients and the controls. Patients who did not have the following were included in the study: a blood transfusion in the previous 3 months, a painful crisis in the previous month, wheezing or an upper respiratory tract infection in the preceding 2 months, treatment other than folic acid, addiction to alcohol or smoking, immunodeficiency, hepatic disease, or heart disease. None of the patients had a history of asthma, recurrent wheezing, use of anti-asthmatic medication, chronic bronchitis, or any pulmonary symptoms that limit daily activities. The diagnosis of asthma was excluded depending on the history of clinical signs and symptoms. All patients had a complete blood workup within 2 weeks of the pulmonary function tests and normal chest X-ray and echocardiographic findings. Patients were considered to have ACS if they had chest pain, fever, and shortness of breath and a new pulmonary infiltration on chest X-ray. The history of ACS was obtained from the patients' medical records. Among 137 adult patients who being followed up in our hospital, 34 patients were found to be eligible for the study according to their medical records and detailed history; three of them had no interest in participating in the study. Nonsmoking, asymptomatic healthy adults, recruited from the general population and matched for age, sex, and race served as controls.

Pulmonary Function Tests

Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1 /FVC ratio, forced expiratory flow rate at 25% to 75% of FVC ($FEF_{25\%-75\%}$), peak expiratory flow rate (PEFR), and total lung capacity (TLC) were measured with a spirometer calibrated daily ($V_{max}22$; SensorMedics, Yorba Linda, CA), using

methane gas, by the same technician according to the criteria of the American Thoracic Society [14]. The best three FEV_1 and FVC measurements were selected. Pulmonary function tests (PFTs) were evaluated as “normal” (TLC, FVC, FEV_1 values [at least 80% of predicted] and FEV_1 /FVC ratio $\geq 70\%$), “obstructive” (FEV_1 /FVC ratio $< 70\%$ and normal or increased TLC), “restrictive” (TLC and/or FVC value $< 80\%$ of predicted together with normal FEV_1 /FVC ratio [at least 70%]), or “mixed” (decrease in FEV_1 /FVC ratio and TLC value) according to the criteria of American Thoracic Society [15].

Diffusion Capacity

Carbon monoxide diffusion capacity was measured with the single breath method using the same spirometer. Diffusion capacity was adjusted according to hemoglobin (Hb) concentration using the following formula: adjusted carbon monoxide diffusion capacity = measured carbon monoxide diffusion capacity/($0.06965 \times Hb$). Values below 80% of predicted values were defined as a “decrease in carbon monoxide diffusion capacity.”

Methacholine Provocation Test

AHR was assessed by a methacholine provocation test (MPT) in patients with SCD and in controls. Methacholine (acetyl-b-methacholine chloride; Sigma-Aldrich Chemie GmbH, Munich, Germany) solution was prepared daily in concentrations between 0.0625 and 16 mg/ml. Solutions were removed from the refrigerator 30 min before testing so they would reach room temperature. Tests were done using the five-breath dosimeter method [16]. The nebulizer with an output of 9 μ l/0.6 s (Spira Electro 2 Inhalation Dosimeter; Respiratory Care Center, Hameenlinna, Finland) was calibrated before the tests. Buffered saline solution was inhaled with five deep breaths after measuring FEV_1 values. The measured FEV_1 value after saline inhalation was used as the reference value. Methacholine was inhaled serially in increasing concentrations at 5-minute intervals. Spirometry was performed 30 and 90 s after each level had been conducted, and the best maneuver was selected. The provocative concentration of methacholine that causes a 20% decrease in FEV_1 (PC20 methacholine) was calculated using the standard formula [16]. The cutoff value for methacholine was accepted as less than 16 mg/ml according to American Thoracic Society guideline [16].

Statistical Analyses

Homogeneities of the variances of the groups were evaluated using Levene's test. The normality of the distribution of the data was confirmed by the Shapiro-Wilk test. While deciding

which statistical methods to use to compare the study groups, the results of the homogeneity and normality tests were used. According to those test results, homogeneous variances and normally distributed patient and control groups were compared using the *t* test, and the results are expressed as mean $\pm$ standard deviation and standard error of the mean. Data not normally distributed and with heterogeneous variances groups were compared using the Mann-Whitney *U* test, and the results are expressed as mean $\pm$ standard deviation, medians, and minimum-maximum values. Spearman's rho correlation coefficient was used for the correlation analysis of variables without a normal distribution. Categorical variables were statistically evaluated with the Pearson χ^2 and Fisher exact test with the Bonferroni correction. The results were expressed as “*n*, %” for categorical variables. The SPSS program (Statistical Product and Services Solutions ver. 13.0; SPSS Inc., Chicago, IL) was used for all statistical analyses. Values of *p* less than 0.05 were considered statistically significant.

Results

There were 31 patients (18 women, 13 men; mean age = 27.48 ± 7.05 years; age range = 18–44 years) with hemoglobin-SS (HbSS) (*n* = 10) and β -thalassemia (HbS β) (*n* = 21) and 31 healthy controls (18 women, 13 men; mean age = 28.00 ± 5.60 years; age range = 18–42 years). There was no significant difference between the two groups with respect to age, height, or weight (Table 1). Twenty patients had a history of ACS and 11 patients had no history of ACS. Ten patients had one ACS episode, seven had two episodes, and three had three episodes.

Lung Volumes and Expiratory Flow Rates

Fifteen patients (48%) had restrictive, 6 (19%) had both obstructive and restrictive, and 4 (13%) had obstructive ventilatory defects. FVC, FEV₁, FEF_{25%–75%}, PEF, and TLC values were significantly lower in patients with SCD than in the controls (Table 2). There was no correlation between the number of ACS episodes and the decrease in

Table 2 Pulmonary function test results in adults with sickle cell disease and in controls

%	Patients with sickle cell disease	Controls	<i>p</i>
FVC	79	99	<0.001
FEV ₁	75	93	<0.001
FEV ₁ /FVC	82	81	0.4
FEF _{25%–75%}	66	83	0.005
PEF	69	93	<0.001
TLC	73	94	<0.001
DLCO	76	88	<0.001

lung function. No significant difference was found between patients with and without a history of ACS with regard to lung volumes and expiratory flow rates.

Diffusion Capacity

The mean Hb value was 9.14 ± 1.43 in patients with SCD. DLCO values adjusted with Hb concentration were significantly lower in patients than in controls (Table 2). There was no significant difference between patients with and without a history of ACS with regard to DLCO. No significant correlation was found between the number of ACS episodes and the decrease in DLCO.

Airway Hyperresponsiveness

The results of the MPT were positive in 15 patients (48%; 11 patients with ACS history and 4 patients without ACS history) and in 5 controls (16%) (*p* = 0.007). The PC20 value was less than 4 mg/ml in 13 (86%) of the patients with a positive MPT. Seven of 16 patients with negative MPT results had no history of ACS and the remaining patients had a history of ACS. There was no significant difference between the patients with and without a history of ACS with respect to MPT positivity. There was a significant correlation between the number of ACS episodes and MPT positivity (*r* = 0.379, *p* = 0.035). The FEF_{25%–75%} value was found to be significantly lower in the patients with a positive MPT result when compared with patients with a negative MPT (*p* = 0.027).

Table 1 Characteristics of the study groups

	Patients with sickle cell disease (<i>n</i> = 31)	Controls (<i>n</i> = 31)
Age	27.48 ± 7.05 (1.26)	28.00 ± 5.60 (1.01)
Height (cm)	166.90 ± 7.72 (1.38)	167.09 ± 8.45 (1.51)
Weight (kg)	60.87 ± 11.21	62.61 ± 12.36
Gender (M/F)	13/18	13/18

Values are mean $\pm$ standard deviation (standard error of the mean)

Discussion

We aimed to assess the pulmonary function of stable adult SCD patients, determine AHR prevalence, evaluate the effects of ACS on lung function, and examine the differences between SCD patients and healthy controls. Our results show that not only a restrictive but also an obstructive pattern may be seen in patients with SCD.

History of ACS does not affect pulmonary function. AHR prevalence is significantly higher in SCD patients than the healthy controls. A significant correlation exists between AHR and recurrent ACS episodes.

It is thought that CSLD develops as a result of recurrent ACS episodes and ends in chronic fibrosis [1–3]. Because of this, restrictive lung defects are frequently seen in adults with SCD [2, 7]. Progressive restrictive lung disease may develop with increasing age, even in the absence of ACS [1]. The most common ventilatory defect seen in children with SCD is airway obstruction [5, 9, 17], whereas there are studies reporting obstructive ventilatory defects [4] in adults and normal spirometry results [18] and restrictive ventilatory defects in children [19].

In a retrospective study that evaluated 63 children with SCD, the rates of obstructive and restrictive diseases were 35 and 8%, respectively [5]. In that study the authors suggested that chronic inflammation initially affects the smaller airways; long-standing inflammation causes lower-airway obstruction in early phases but may lead to fibrosis in later phases [5]. This may explain the increased prevalence of restrictive ventilatory defects in adults. Another study supporting this view is from Sylvester et al. [20] who suggest that the restrictive defect becomes obvious with increasing age in children with SCD. Lung dysfunction, especially the restrictive type, may develop due to various factors such as pulmonary fibrosis (probably due to recurrent ACS attacks, infections, and vascular infarcts) or to unproportional development of the thorax and the extremities [5, 6]. In our study we determined both obstructive and restrictive defects in six patients and only obstructive defects in four patients; therefore, we suggest that the restrictive ventilatory defect is not the only prominent feature of adult patients with SCD.

Because conventional PFTs are not satisfactory for determining diseases affecting the pulmonary vascular structures, it has been reported that exercise oximetry and DLCO measurements may be helpful during follow-up of chronic progression of pulmonary complications [3]. Many studies have shown that DLCO decreases in patients with SCD [6, 7, 13]. In our study, the mean DLCO value was significantly lower in adult SCD patients than in healthy controls. In an autopsy study, scarring, arteriolar occlusion with periarteriolar fibrosis, unresolved pneumonia, and alveolar wall necrosis, all leading to a decrease in lung volume, were detected [21]. These changes in the lungs of patients with SCD may explain the decrease in DLCO. On the other hand, Santoli et al. [4] found a nonsignificant increase in DLCO in patients with a history of ACS and suggest that this may be due to the increase in pulmonary blood volume.

An increased incidence of AHR was demonstrated by a positive response to bronchodilators, MPT, or indirect

provocation tests (cold air, exercise) in patients with SCD [5, 10–13], whereas the exact relationship between the increased incidence of AHR and episodes of ACS has not been elucidated. In SCD, a constant low level of inflammation develops due to abnormal adhesion of the sickled erythrocytes to the vascular endothelium [1, 22]. In some studies, increased AHR has been shown to exist in SCD patients who do not have asthma [5, 12]. In a study of Leong et al. [12], the prevalence of AHR was 83 and 64% in children with SCD with and without asthma, respectively; while Vendramini et al. [13] found AHR in 31% of the adults with SCD. In our study, the prevalence of AHR was 48%, and a significant relationship between AHR and recurrent episodes of ACS was shown in adults with SCD. Because our patients were not symptomatic, AHR might have been due to the presence of underlying subclinical asthma or a nonspecific finding of inflammation in the lung (airway and parenchyma). Depending on the relationship between AHR and $FEF_{25\%-75\%}$ shown in our study, we suggest that the inflammation is present mainly in the small airways. We believe that the prevalence of AHR is high in patients with SCD; on the other hand, to reach a final decision regarding the relation between SCD and reversible airway obstruction, additional studies are needed.

A significant relationship between asthma and ACS has been reported [10, 23, 24]. This relationship is attributed to vascular leakage and increased mediator release during the ACS episode; these processes are reported to cause an increase in AHR and, hence, asthma [24]. The results of some other studies support the idea that asthma exacerbation could lead to an ACS episode in patients with SCD [10, 23]. In SCD, the sickled erythrocytes, with their increased burden of oxidants, threaten the integrity of the endothelium to which they adhere [1]. Peripheral circulating erythrocytes carrying very late antigen-4 adhere to the endothelial cells by binding specifically to endothelial vascular cell adhesion molecules (VCAM) in patients with SCD. Endothelial VCAM expression is regulated by inflammatory cytokines such as interleukin-1 (IL-1), IL-4, and tumor necrosis factor- α [1, 25]. As a result, abnormal adhesion of the sickled erythrocytes to the vascular endothelium leads to a vaso-occlusive crisis. Depending on these factors, it is suggested that oxidant products and inflammatory mediators [26, 27] that appear during an asthma exacerbation may cause a vaso-occlusive crisis by enabling the adherence of the erythrocytes to the vascular endothelium [1, 25]. On the other hand, hypoxemia that occurs during an asthma exacerbation may trigger an ACS episode by increasing sickling in the pulmonary vasculature.

In a study by Knight-Madden et al. [11], asthma and AHR were seen more frequently in children with SCD than in healthy controls. In that study, the relationship between atopy and ACS episodes was reported, but no significant

difference was found between the patients and the controls with respect to atopy. Therefore, it is thought that these patients were misdiagnosed as having asthma and that, in fact, they had AHR. In another study [23], children with SCD who had a previous ACS episode were found to have a higher ratio of antiasthmatic medication than those without an ACS episode, and the diagnosis of asthma was before the first ACS episode in the former group. These two studies suggest that an early and effective antiasthmatic therapy may decrease the morbidity related to SCD. Our study is significant because it is the first study to demonstrate the relationship between AHR and recurrent ACS episodes in adult SCD patients without asthmatic symptoms. We found the ratio of MPT positivity to be significantly higher in adult patients with recurrent ACS episodes than in patients with a single or no ACS episode ($p = 0.023$). In addition, MPT positivity increased with increasing number of episodes. We suggest that recurrent episodes of ACS may lead to development of AHR, or, conversely, the presence of AHR may trigger ACS episodes. If the second hypothesis is valid, this point of view will support the results of the former two studies [11, 23].

In conclusion, the lung function of adult patients with SCD is significantly different from that of healthy race matched controls of similar age. Prevalence of AHR was significantly higher in SCD patients without asthmatic symptoms than in the healthy controls. If future studies can verify the significant correlation we found between AHR and recurrent ACS episodes, routine use of anti-inflammatory controlling agents should be considered for decreasing pulmonary morbidity in SCD patients, even in the absence of asthmatic symptoms. Multicenter prospective studies with large study populations are needed to determine the progression of pulmonary function from childhood to adulthood in SCD and to understand the relationship between asthma, AHR, ACS, and lung dysfunction.

Acknowledgment The authors thank O. Yaylagulu for his technical assistance and S. Yumak for her secretarial support.

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