Correlates of Pulmonary Function in Children with Sickle Cell Disease and Elevated Fetal Hemoglobin

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Significance of the Study
- Pulmonary function was studied in pediatric patients with sickle cell disease (SCD) in Kuwait and its correlation with selected clinical, laboratory, and hematological parameters was determined.
- The patients demonstrated poorer lung function, especially forced expiratory volume in 1 s, compared to normal controls. There was no correlation with many of the clinical and laboratory parameters studied.
- This showed that pulmonary function abnormalities could develop early in Kuwaiti patients with SCD and the pathogenesis requires further investigation.

Keywords
Pulmonary function · Sickle cell disease · Children · Fetal hemoglobin

Abstract
Objective: The current study was carried out to compare pulmonary function tests (PFTs) in pediatric Kuwaiti sickle cell disease (SCD) patients to age-matched normal controls and to investigate the association of PFTs with selected clinical and laboratory parameters. Subjects and Methods: There were 38 patients with SCD and 36 controls in the study. The patients were recruited from the Pediatric Hematology Clinics of Mubarak Al-Kabeer and Al-Amiri Hospitals, Kuwait, and were studied in steady state. The controls were healthy, non-sickle cell siblings of the patients. Forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), total lung capacity, and other PFT parameters were obtained using a constant-volume, variable-pressure, body plethysmograph. Hemoglobin, fetal hemoglobin, serum bilirubin, and lactate dehydrogenase were determined using standard methods. Results: The mean ages of the patients and controls were 10.5 ± 3.2 and 10.5 ± 3.5 years, respectively. The FEV₁% predicted of 84.1 ± 15.4% among the patients was significantly lower than the 92.1 ± 11.8% in the controls (p = 0.003). The FVC% predicted was also significantly lower (p = 0.022) in the patients than in the controls, although the values were generally within the normal range. There was no association of FEV₁ with pain phenotype, acute chest syndrome (ACS), or blood transfusions. Also, there was no
significant correlation with reticulocytes, bilirubin, or lactate dehydrogenase. **Conclusions:** In this study, changes in PFT, especially FEV₁, developed early in the SCD patients. There was no demonstrable association with frequent vaso-occlusive crisis, ACS, and other variables. Hence, there is a need for follow-up studies with serial PFTs to identify vulnerable patients, who might need intervention to prevent early mortality.

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**Introduction**

Cardiopulmonary complications are harbingers of grave morbidity in sickle cell disease (SCD), and several studies have shown an association of elevated tricuspid regurgitant jet velocity and reduced forced expiratory volume in 1 s (FEV₁) with early mortality in adult patients [1–3]. The factors predisposing SCD patients to cardiorespiratory complications include recurrent acute chest syndrome (ACS) [4, 5], pulmonary hypertension [6–8], wheezing, and asthma [9–11]. The underlying pathophysiological mechanism involves progressive vasculopathy, instigated by free heme, which sets off a cascade of inflammatory processes, culminating in nitric oxide depletion with eventual endothelial constriction and smooth muscle hyperplasia [12–14].

Although SCD follows a relatively mild course among Kuwaiti children, a subset exists with frequent vaso-occlusive crisis with consequent complications [15]. There has been only one previous study of pulmonary function in these patients and it showed early signs of abnormality that were unrelated to their level of anemia [16]. However, other genetic and environmental factors associated with pulmonary function were not investigated. The reported prevalence rates of wheeze ever, current wheeze, and physician-diagnosed asthma among otherwise normal 13- and 14-year-old Kuwaiti schoolchildren were 13.4, 7.6, and 15.6%, respectively [17, 18]. However, there are no reports among younger children or patients with SCD.

Therefore, the present study was designed to investigate the influence of asthma, previous ACS, and frequency of blood transfusions on pulmonary function tests (PFTs) in a group of Kuwaiti children with SCD in comparison to age-matched normal controls, and to investigate the relationship of markers of hemolysis (reticulocyte count, serum lactate dehydrogenase, and bilirubin) and the use of hydroxyurea with PFT among these patients.

### Subjects and Methods

This was a cross-sectional study involving 38 SCD patients and 36 controls. Assuming an expected 70% of SCD patients and 90% of controls having normal PFT, the power calculation gave a sample size of at least 35 in each arm. The patients were consecutive patients being followed in the Pediatric Hematology Clinics of Mubarak Al-Kabeer and Amiri Hospitals, Kuwait, from October 2014 to November 2015. Inclusion criteria were follow-up of at least 1 year in the clinic, steady state (no acute illness or blood transfusion in the preceding 6 weeks) at the time of the study, and no evidence of any other chronic disease that could affect pulmonary function. The patients were fully characterized using cation-exchange high-performance liquid chromatography and β²-haplo-type and α-globin genotype, determined using allele-specific oligonucleotide hybridization as previously described [15]. The controls were healthy, non-sickle cell siblings of the patients. Since blood was not collected from the controls, their hemoglobin (Hb) genotypes were not confirmed, but they had all been screened as part of family studies and it was known that they did not have SCD. Written, informed consent was obtained from the parents of all the study subjects. Human Research Ethics Committees of the Faculty of Medicine, Kuwait University and the Ministry of Health Kuwait approved the study.

The patients’ charts were reviewed to document the following: any regular medications with their doses, frequency of blood transfusion, details and diagnoses of previous hospitalizations, and administration of hydroxyurea if the patients had >3 hospitalizations due to severe pain crisis in a year. The average number of hospital admissions for vaso-occlusive crisis in the preceding 2 years was noted and classified as mild (0–1), moderate (2–3), and severe pain (>3) phenotypes. ACS was defined as acute respiratory symptoms associated with fever, chest pain, clinical signs of parenchymal lung involvement, and new pulmonary infiltrates on chest radiography.

The parents of the subjects and/or the subjects older than 7 years were interviewed using a modified American Thoracic Society Division of Lung Diseases’ Questionnaire (ATS-DLD-78-C) [19] for details of any previous respiratory symptoms and doctor-diagnosed wheezing or asthma and allergies. The questionnaire was translated into Arabic, and was pretested and validated in a group of 10 Kuwaiti subjects. It was administered by a native Arabic-speaking doctor member of the research team (A.F.A.).

A sample of 5–10 mL of blood was obtained by venipuncture from the patients. An electronic cell counter was used to obtain complete blood count, including reticulocytes. Cation-exchange high-performance liquid chromatography was done to confirm the Hb genotype. DNA was extracted and the Hb subunit gamma-2 (HGB2) Xmn-1 polymorphism was determined as previously described. Total serum bilirubin and lactate dehydrogenase were determined using routine methods.

The patients were weighed (kilograms) and height measured (centimeters), and pulmonary function was measured in the sitting position using a constant-volume, variable-pressure, body plethysmograph (version 4.5; Erich Jaeger Master Laboratory GmbH, Höchberg, Germany), based on the American Thoracic Society (ATS) guidelines [20]. The variables analyzed included forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), the ratio of FEV₁/FVC%, peak expiratory flow, mid flow rate (maximal mid-expiratory flow 75/25), total lung capacity, and residual
volume. The pulmonary carbon monoxide transfer factor was assessed using the single breath method and corrected for Hb level to obtain the diffusion capacity of the lung for carbon monoxide. The data were expressed as a percentage of the predicted values, based on the age and height of each subject. The PFTs were carried out and interpreted by a pulmonologist with years of experience in pediatric care. The patterns were classified as normal, obstructive, restrictive, nonspecific, or mixed based on ATS guidelines [21].

Statistical Analysis
Data were analyzed using Statistical package for the Social Sciences (SPSS), version 23 (IBM Corp., Armonk, NY, USA) and are presented as means ± SD. The differences between means of continuous data were tested for statistical significance using the Student t test, while categorical data were tested with the χ² or Fisher test. Pearson’s correlation coefficients were computed to test the relationship between sets of data. Multiple regression analysis was also carried out. The level of significance was set at p < 0.05.

Results
The summary of the PFT and anthropometric data on the patients and controls are shown in Table 1. The mean ages of patients and controls were similar (10.5 ± 3.2 and 10.5 ± 3.1 years, respectively). While the height and weight were lower among the patients than the controls, the differences were not statistically significant (p > 0.05). The mean FEV₁ of 84.1 ± 15.4% predicted among the patients was significantly lower than the 92.1 ± 11.8% in the controls (p = 0.003). The FVC% predicted was also significantly lower (p = 0.022), but the difference in the FEV₁/FVC ratio just fell short of significance (p = 0.051). The other PFT values were similar in both groups.

Of the 35 SCD patients in whom the frequency of pain crisis was recorded, 22 (62.9%) had a mild phenotype, while 7 (20.0%) were moderate, and 6 (17.1%) were severe. Among those in whom the information was provided, 8 of 35 (22.9%) had at least 1 episode of ACS in the preceding 2 years. No patient was on chronic transfusion. Nineteen (52.7%) of the 35 SCD patients were on hydroxyurea. Only 2 patients (5.3%) gave a history of doctor-diagnosed asthma, and 4 (10.5%) had previous episodes of wheezing. Pain phenotype, past ACS, and blood transfusion history also did not influence the mean values significantly. Since only 2 patients gave a history of doctor-diagnosed asthma, this was not subjected to further analysis.

Of the 38 patients with SCD, 19 (50%) were Hb Sβthalassemia (Sβthal), 17 (44.7%) Hb SS, and 2 (5.3%) Hb SD. The hematological parameters among the SCD patients are shown in Table 2. There were no significant differences when these were compared between the SS patients and patients with other genotypes. When the mean PFT values were compared among the SS and the Sβthal patients, there were no significant differences (data not shown).

α-Globin genotypes were available in 24 patients, 8 (33.3%) of whom had an α-thal trait; 4 with –α/αα and 4 with –α/–α. However, when the PFT parameters were compared between the 8 with the α-thal trait and those without, there were no significant differences. All the 19 SS patients but 3 were homozygous for the Xmn-1 T allele, while all the Sβthal and SD patients were heterozygous. Since there were no patients who were negative for this allele, its influence on PFTs was not investigated.

The correlation coefficients between the FEV₁ and anthropometric data and markers of hemolysis (Table 3) showed a strong correlation with height (r = 0.9, p < 0.001) and weight (r = 0.8, p < 0.001) but not with Hb, fetal Hb, retics, total bilirubin, and lactate dehydrogenase. The patterns with other PFTs were similar, except that Hb correlated well with the FEV₁/FVC ratio (r = 0.4, p = 0.02). Linear regression analysis of the independent variables against each PFT as the dependent variable did not show a significant correlation.

The distribution of PFT patterns among the patients were as follows: normal: 27 (71.5%), obstructive: 4 (10.5%), nonspecific: 5 (13.2%), and restrictive: 1 (2.6%). Among the controls, 33 (91.7%) were normal and the re-

Table 1. Anthropometric and pulmonary function data on study participants

|                  | SCD (n=38) | Controls (n=36) | All (n=74)  |
|------------------|------------|----------------|-------------|
| Age, years       | 10.5±3.2   | 10.5±3.1       | 10.5±3.1    |
| Weight, kg       | 38±19.5    | 40.3±16.1      | 39.5±17.8   |
| Height, cm       | 139.0±16.0 | 143.9±16.3     | 141.4±16.2  |
| FEV₁, %          | 84.1±15.4* | 92.1±11.8      | 88.0±14.2   |
| FVC, %           | 87.2±13.8** | 93.4±11.6     | 90.2±13.1   |
| FEV₁/FVC         | 85.8±6.9   | 88.4±5.1       | 87.1±6.2    |
| TLC, %           | 99.0±11.3  | 99.7±6.1       | 99.4±9.1    |
| DLCO, %          | 84.4±24.4  | 87.1±19.9      | 85.8±22.1   |

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, diffusion capacity of the lung for carbon monoxide; SCD, sickle cell disease; TLC, total lung capacity. *p = 0.003, **p = 0.022, statistically significant.
remaining 2 (5.6%) had nonspecific patterns. The difference in the distribution was not statistically significant, using the $\chi^2$ test ($\chi^2 = 6.835$, df = 3, $p = 0.077$).

**Discussion**

In this study, only 2 patients (5.3%) reported a positive history of asthma. This is surprising given the high frequency of asthma in the general pediatric population [17, 18]. On the other hand, almost 30% of patients in the study had at least 1 episode of ACS, but it tended to be mild and had no influence on PFTs. The low prevalence of asthma and the mild nature of ACS in this study could probably be reflective of the SCD phenotype among Kuwaiti pediatric patients, which confirmed the premise suggested by Koumbourlis [22] that severity, and not frequency of ACS, is a better predictor of pulmonary dysfunction in SCD patients.

It is, however, pertinent to note that in spite of the mild phenotype of the disease, there was still a significant reduction in the PFT parameters, especially FEV$_1$ in the patients compared to normal controls. While most of the patients and controls had normal respiratory patterns, 26.3% of the SCD patients had abnormal or nonspecific changes but only 5.6% of the controls had nonspecific patterns. There was also no clear association of FEV$_1$ or other PFTs with individual markers of hemolysis. Similarly, the frequency of pain episodes, blood transfusions, or hydroxyurea use did not have an influence on the PFT's. This confirms that irrespective of the SCD phenotype, pulmonary function changes occur early in SCD [22].

In the present study, pulmonary function changes occurred early and an obstructive pattern was more common in the age group studied. Although airway hyperreactivity and asthma contribute to the decline in respiratory function, in SCD [23–25], other factors include ACS.

| Table 2. Hematological parameters among SS, Sβ-thal, and SD patients |
|---------------------------------|----------------|----------------|----------------|----------------|
|                                 | SS ($n = 16$) | Sβ-thal ($n = 19$) | SD ($n = 2$) | All ($n = 38$) |
| Hb, g/dL                        | 10.1 ± 0.8    | 9.7 ± 1.3       | 11.5 ± n.a.   | 9.9 ± 1.1     |
| Hct, %                          | 30.4 ± 2.8    | 29.9 ± 4.1      | 34.0 ± n.a.   | 30.2 ± 3.5    |
| HbF, %                          | 21.1 ± 5.4    | 23.7 ± 10.3     | 36.2 ± n.a.   | 22.9 ± 9.4    |
| WBC, ×10$^9$/L                  | 10.2 ± 6.2    | 9.5 ± 5.3       | 12.5 ± 3.3    | 10.2 ± 5.6    |
| Retics, %                       | 5.1 ± 2.3     | 4.7 ± 2.9       | 3.1 ± n.a.    | 4.8 ± 2.6     |
| Bilirubin, mmol/L               | 34.4 ± 12.7   | 40.3 ± 18.9     | 28.1 ± 8.3    | 36.0 ± 15.5   |
| LDH, IU/L                       | 423.0 ± 559.4 | 290.1 ± 73.2    | n.a.          | 356.5 ± 396.7 |

Hb, hemoglobin; Hct, hematocrit; HbF, hemoglobin F; WBC, white blood cells; LDH, lactate dehydrogenase; Sβ-thal, hemoglobin Sβ-thalassemia; SS, hemoglobin SS; SD, hemoglobin SD; n.a., not applicable.

| Table 3. Correlation between pulmonary function parameters, anthropometric data, and markers of hemolysis |
|---------------------------------|--------|--------|--------|--------|--------|--------|
| PFT    | Age     | Height | Weight | HbF    | Hb     | Retics  | Total bilirubin | LDH |
| FEV$_1$ | 0.17 (0.32) | 0.05 (0.76) | 0.12 (0.49) | 0.09 (0.61) | 0.02 (0.90) | 0.01 (0.94) | -0.10 (0.63) | -0.26 (0.20) |
| FVC    | 0.05 (0.75) | -0.02 (0.89) | 0.03 (0.86) | 0.13 (0.46) | -0.16 (0.34) | 0.09 (0.64) | 0.00 (0.99) | -0.35 (0.08) |
| FEV1/FVC| -0.04 (0.81) | -0.16 (0.35) | 0.02 (0.93) | -0.01 (0.95) | 0.23 (0.16) | -0.15 (0.41) | -0.27 (0.20) | 0.17 (0.41) |
| TLC    | 0.00 (0.99) | -0.03 (0.87) | -0.07 (0.68) | 0.15 (0.40) | -0.05 (0.78) | 0.03 (0.86) | -0.02 (0.92) | -0.02 (0.91) |
| DLCO   | 0.23 (0.22) | 0.10 (0.58) | 0.02 (0.91) | 0.086 (0.66) | 0.26 (0.16) | -0.07 (0.72) | -0.05 (0.83) | 0.10 (0.64) |

The correlation coefficient is provided, with the $p$ value in parenthesis. PFT, pulmonary function test; Hb, hemoglobin; HbF, hemoglobin F; LDH, lactate dehydrogenase; FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusion capacity of the lung for carbon monoxide.
and pulmonary hypertension [5, 6, 9]. However, quite often, no predisposing factors can be identified other than the chronic vasculopathy that characterizes the disease [26]. Pulmonary function abnormalities develop early in the disease and while the respiratory pattern may be normal or obstructive, it is mostly associated with a restrictive pattern in adults [26].

The differences noticed among Kuwaiti SCD patients compared to those from other parts of the world may be because the former have high fetal Hb levels that could be up to 30% in the first 2–4 years of life [27]. Because of this and other factors, many are asymptomatic in early childhood. Complications of SCD like dactylitis, leg ulcers, priapism, and stroke are uncommon [15]. This may also explain why wheezing and asthma are not prominent features among our pediatric SCD patients. By the time the patients reach adulthood, the disease may be severe with debilitating multi-organ complications [28]. This indicates that there is a transition from the mild phenotype in childhood to a severe picture later in life. It is, therefore, pertinent to monitor pediatric patients to identify those at risk and in need of early intervention.

In a previous study from our center, SCD patients had the lowest PFT parameters when compared to normal controls and patients with HbH disease [16]. In a study from India, which also looked at children with SCD and elevated fetal Hb levels, Purohit et al. [29] reported significantly lower values of FEV\textsubscript{1} and FVC in the patients compared to healthy controls, similar to the results from the present study. The Indian study, however, did not comment on the prevalence of asthma, although 11% had previous episodes of ACS.

The limitations of this study included (a) its cross-sectional design, which gave only a snapshot of the pulmonary status of the patients (a prospective, longitudinal study would be more desirable to appreciate the progression of pulmonary decline), and (b) the small sample size of the subgroups.

**Conclusion**

In this study, there was a significant reduction in PFT parameters, especially FEV\textsubscript{1}, in the patients compared to normal controls, although they remained generally within the normal range. There was also no clear association of FEV\textsubscript{1} or other PFTs with individual markers of hemolysis. Similarly, the frequency of pain episodes, blood transfusions, or hydroxyurea did not influence the PFTs.

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**References**

1. Platt OS, Brambilla DJ, Rosse W, et al: Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 1994; 330:1639–1644.
2. Kassim AA, Payne AB, Rodeghier M, et al: Low forced expiratory volume is associated with earlier death in sickle cell anemia. Blood 2015;126:1544–1550.
3. Chaturvedi S, Labib Ghafuri D, Kassim A, et al: Elevated tricuspid regurgitant jet velocity, reduced forced expiratory volume in 1 sec, and mortality in adults with sickle cell disease. Am J Hematol 2017;92:125–130.
4. Desai PC, Ataga KI: The acute chest syndrome of sickle cell disease. Expert Opin Pharmacother 2013;14:991–999.
5. Sylvester KP, Patey RA, Milligan P, et al: Impact of acute chest syndrome on lung function of children with sickle cell disease. J Pediatr 2006;149:17–22.
6. Gladwin MT, Sachdev V, Jison ML, et al: Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N Engl J Med 2004;350:886–895.
7. Castro O, Gladwin MT: Pulmonary hypertension in sickle cell disease: mechanisms, diagnosis, and management. Hematol Oncol Clin North Am 2005;19:881–896, vii.
8. Castro O, Minniti CP, Nouraei M, et al: Pulmonary hypertension in sickle cell disease. N Engl J Med 2011;365:1646; author reply 1648–1649.
9. Boyd JH, Macklin EA, Strunk RC, et al: Asthma is associated with acute chest syndrome and pain in children with sickle cell anemia. Blood 2006;108:2923–2927.
10. Knight-Madden JM, Forrester TS, Lewis NA, et al: Asthma in children with sickle cell disease and its association with acute chest syndrome. Thorax 2005;60:206–210.
11. Nordness ME, Lynn J, Zacharisen MC, et al: Asthma is a risk factor for acute chest syndrome and cerebral vascular accidents in children with sickle cell disease. Clin Mol Allergy 2005;3:2.
12. Kato GJ, Hebbel RP, Steinberg MH, et al: Vasculopathy in sickle cell disease: biology, pathophysiology, genetics, translational medicine, and new research directions. Am J Hematol 2009;84:618–625.
13. Morris CR, Gladwin MT, Kato GJ, et al: Nitric oxide and arginine dysregulation: a novel pathway to pulmonary hypertension in hemolytic disorders. Curr Mol Med 2008;8:620–632.
14. Wood KC, Hsu LL, Gladwin MT: Sickle cell disease vasculopathy: a state of nitric oxide resistance. Free Radic Biol Med 2008;44:1506–1528.
15 Adekile AD, Haider MZ: Morbidity, beta S haplotype and alpha-globin gene patterns among sickle cell anemia patients in Kuwait. Acta Haematol 1996;96:150–154.
16 Hijazi Z, Onadeko BO, Khadadah M, et al: Pulmonary function studies in Kuwaiti children with sickle cell disease and elevated Hb F. Int J Clin Pract 2005;59:163–167.
17 Abal AT, Ayed A, Nair PC, et al: Factors responsible for asthma and rhinitis among Kuwaiti schoolchildren. Med Princ Pract 2010;19:295–298.
18 Owayed NA, Behbehani N, Al-Momen J, et al: Changing prevalence of asthma and allergic diseases among Kuwaiti children. An ISAAC Study (Phase III). Med Princ Pract 2008;17:284–289.
19 Ferris BG: Epidemiology Standardization Project (American Thoracic Society). Am Rev Respir Dis 1978;118:1–120.
20 Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med 1995;152:1107–1136.
21 Pellegrino R, Viegi G, Brusasco V, et al: Interpretative strategies for lung function tests. Eur Respir J 2005;26:948–968.
22 Koumourlis AC: Acute chest syndrome, asthma, and lung function in sickle cell disease. Which is the chicken, and which is the egg? Ann Am Thorac Soc 2016;13:1212–1214.
23 Cohen RT, Klinge ES, Strunk RC: Sickle cell disease: wheeze or asthma? Asthma Res Pract 2015;1:14.
24 Galadanci NA, Liang WH, Galadanci AA, et al: Wheezing is common in children with sickle cell disease when compared with controls. J Pediatr Hematol Oncol 2015;37:16–19.
25 Musa BM, Galadanci NA, Rodeghier M, et al: Higher prevalence of wheezing and lower FEV1 and FVC percent predicted in adults with sickle cell anemia: a cross-sectional study. Respir Med 2017;22:284–288.
26 Koumourlis AC: Lung function in sickle cell disease. Paediatr Respir Rev 2014;15:33–37.
27 Adekile A, Al-Kandari M, Haider M, et al: Hemoglobin F concentration as a function of age in Kuwaiti sickle cell disease patients. Med Princ Pract 2007;16:286–290.
28 Alsultan A, Alabdulaali MK, Griffin PJ, et al: Sickle cell disease in Saudi Arabia: the phenotype in adults with the Arab-Indian haplotype is not benign. Br J Haematol 2014;164:597–604.
29 Purohit R, Rao SS, Goyal JP, et al: Pulmonary function tests in sickle cell disease. Indian J Pediatr 2016;83:783–786.