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Original Article

Sleep disorders and endothelial dysfunction in children with sickle cell anemia

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ABSTRACT

Objective: We sought to assess sleep characteristics in children with sickle cell anemia (SCA) to investigate the possible association between sleep-related disorders and parameters of endothelial function.
Methods: Sleep Disturbance Scale for Children (SDSC) and endothelial function variables (flow-mediated dilatation using brachial artery ultrasound, cytokines) were compared in children with SCA and healthy children aged 6–18 years.
Results: Flow-mediated dilation (FMD) median (IQR) values were 10.1 (6.4; 14) *vs* 16.9 (12; 26.4), in the SCA and comparison groups ($p = 0.001$), respectively. Associations were found between difficulty initiating and maintaining sleep as well as IL-12p70 ($rs = 0.3$, $p = 0.03$) and IL-1b ($rs = 0.4$, $p = 0.01$); disorders of excessive sleepiness and TNF-a ($rs = 0.3$, $p = 0.02$), IL-6 ($rs = 0.3$, $p = 0.03$), e IL-17A ($rs = 0.3$, $p = 0.04$), and disorders of arousal with IL-6 ($rs = 0.3$, $p = 0.04$). Regarding SDSC subscales, the score for sleep breathing disorders was higher in the SCA group than in the comparison group [6.7 (3.4) vs. 5.2 (2.7), $p = 0.04$]. Sleep breathing disorders, disorders of excessive sleepiness and sleep hyperhidrosis scores were also higher among those SCA patients who were using hydroxyurea than those with SCA who were not using hydroxyurea [7.4 (3.9) *vs*. 5.9 (2.9); $p = 0.026$; 7.5 (3.5) *vs*. 6.9 (3); $p = 0.028$; 4.3 (3.1) *vs*. 3.9 (2.6); $p = 0.044$, respectively].
Conclusion: The SCA group presented higher sleep-disordered breathing scores and lower FMD values. Patients with SCA using hydroxyurea exhibited a higher frequency of scores on SDSC subscales. A positive correlation was found between SDSC subscales and cytokines.

1. Introduction

Sickle cell disease (SCD) is an inherited disorder in which sickle cell anemia (SCA) is one of the most common monogenetic disorders with a global distribution [1]. It is autosomal recessive, characterized by a mutation in the β-globin gene. This mutation makes erythrocytes less flexible, reduces their affinity for oxygen and distorts them, resulting in the consequent impairment of tissue oxygenation and the development of vascular occlusion and hemolysis; all of which accounts for the broad scope of clinical manifestations of this disease [2–4]. The hypoxia-normoxia cycle is associated with acute and chronic inflammation, which increases the expression of critical adhesion molecules on red blood cells, endothelium, leukocytes, and platelets, leading to vascular dysfunction, vaso-occlusion, tissue ischemia, and reperfusion, with infarction of virtually any organ system [2,5–7].

In SCA, the involvement of the vascular endothelium in the inflammatory response induces the abnormal expression of adhesion molecules, which are related to endothelial activation via inflammatory stimuli. Cytokines [tumor necrosis alpha (TNF-α) and interleukin 1 (IL-1))], and thrombin are among the biological

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modifying factors that trigger endothelial activation during vaso-occlusive crises [8,9].

Endothelial dysfunction (ED) might be induced by sickle cells and secondary endothelial inflammatory response. Nitric Oxide (NO) originates in the endothelium and regulates vasomotor tone in response to variations in wall stress [10–13]. Measurement of flow-mediated dilation in the brachial artery has been generally used to assess endothelial function in a wide range of diseases, including SCD. Abnormalities of endothelium-dependent vasodilation in conduit vessels and changes in vascular wall properties have been demonstrated in adults with SCD [11], yet there are currently few data on children with SCD [14]. In young adult patients with sickle cell anemia, Blum et al., demonstrated that FMD was lower in patients with sickle cell disease in crisis than those in steady state and that both were lower than FMD in controls. Furthermore, they showed heightened levels of biomarkers known to confer an increased risk of endothelial dysfunction, inflammation, and endothelial cell activation [11].

Increase in proinflammatory cytokines (C-reactive protein, tumor necrosis factor alpha, interleukin 6, and interleukin 10) in adult Obstructive Sleep Apnea (OSA) patients, and high-sensitivity C-reactive-protein in pediatric OSA patients supports the hypothesis of an association between the apnea-hypopnea index (AHI) and inflammatory cytokine levels [15]. Setty et al., evaluated sleep in 37 children with sickle cell disease and found a positive correlation between red cell adhesion to endothelium, platelet, and leukocyte activation markers [16]. Data in the literature have pointed to some significant problems related to sleep among children with SCA, including sleep-disordered breathing [17]. Upper airway obstruction might result in hypoventilation with consequent hemoglobin desaturation during sleep, contributing to the development of hypoxemia, hypercapnia, and acidosis that in turn might induce sickle cell hemoglobin polymerization; thus potentially inducing the occurrence of a vaso-occlusive crisis (VOC) [18]. Therefore, SCA and OSA share some molecular pathways that might contribute to the clinical manifestations in these patients [19].

Valrie et al., prospectively assessed children with SCA and found that they experienced frequent night awakenings, difficulty falling asleep, and daytime sleepiness [20]. SCD and sleep-disordered breathing share common pathophysiological pathways, most notably recurrent episodes of hypoxia-reoxygenation, decreased nitric oxide bioavailability, endothelial dysfunction, and chronically increased pro-inflammatory signaling pathways. Knowing of few reports on the association between sleep-disordered breathing and ED among children and adolescents with SCA [16], we hypothesized that there is a relationship between these sleep-related disorders and SCA. The present study aimed to investigate the possible association between sleep-related disorders and ED in children with SCA who either are using or not using hydroxyurea, utilizing the Sleep Disturbance Scale for Children (SDSC) to describe their sleep characteristics.

2. Materials and methods

2.1. Patient selection and descriptions

The present study is an analytical, cross-sectional study that included a comparison group. The eligible population included children with HbSS aged six years or older and younger than 18 who were cared for on a regular basis at a reference pediatric hematology outpatient clinic, as well as age-matched healthy children who were referred by the general preventive pediatric outpatient clinic. Both clinics were part of the Professor Edgard Santos University Hospital Complex, Federal University of Bahia, Brazil; the patients were recruited from April 2014 to June 2015. The study was designed with 80% power to detect a correlation of 0.4 between SRD symptoms and FMD. Considering a type I error of 0.05 and a type II error of 0.20, 47 participants per group were required. However, to achieve the study's objectives and to include the possibility of a 10% loss of subjects, the final sample comprised 122 participants. The case group included 60 children with HbSS (homozygous) diagnosed by hemoglobin (Hb) electrophoresis and/or high-performance liquid chromatography. These children had experienced no acute events (VOC, acute chest syndrome [ACS] and infectious conditions) in the preceding month, had not been treated with antibiotics in the past month and were either using or not using HU. Children with a prior cerebrovascular accident or blood transfusion within the preceding three months were excluded. The comparison group included 62 healthy children of the same age who showed no signs of acute or chronic infection and had normal Hb electrophoresis results. The following exclusion criteria were used: other hemoglobinopathies, sickle cell trait (HbS), associated genetic syndromes, obesity, diabetes mellitus, hypercholesterolemia, blood transfusions within the past three months, acute health events in the past month, cardiovascular disease, and lung disease. Children with confirmed diagnosis of obstructive sleep apnea syndrome (OSAS) under treatment with either drug therapy or continuous positive pressure in the airways were excluded.

This study was approved by the Research Ethics Committee of the Bahiana School of Medicine and Public Health (No. 33705714.3.0000.5544), and the study protocol complied with the provisions of the Declaration of Helsinki. Informed consent was obtained from all of the children's parents or guardians and from the children who were mature enough to understand the study's goals.

2.2. Clinical evaluation

The medical records of the subjects were reviewed for demographic data; a questionnaire was used to obtain information regarding the number of VOCs, the number of patients who were sent to an urgent-emergency care unit due to VOC, the number of hospitalizations for VOC, the number of transfusions, and episodes of acute chest syndrome (ACS). These data were all evaluated over the last year after inclusion in the study. The length of stay for ACS (days), as well as the occurrence of osteomyelitis, priapism, osteonecrosis, splenic sequestration, and splenectomy were also included, along with the number of transfusions until the initiation of the study, and HU use or non-use. The physical examination included weight and height to calculate body mass index (kg/m^2); BMI was expressed according to the WHO z-score system [21]. Systolic and diastolic blood pressures were measured. Daytime oxyhemoglobin arterial saturation (SpO_2) was assessed with a pulse oximeter (Onyx(r) II 9650 Bluetooth; Nonim Medical Inc., Plymouth, MN, USA) for 10 min with the children sitting and in a state of rest immediately after arriving at the clinic on the day of entry in the study.

2.3. Assessment of sleep characteristics

Excessive daytime sleepiness was assessed using the modified Epworth Sleepiness Scale (ESS) [24], comprising eight questions assessing the odds of falling asleep in various common daily life situations. A total score $\geq$10 indicates daytime sleepiness. The Sleep Disturbance Scale for Children (SDSC) [25,26], which assesses behavior and sleep-related disorders over the past six months, was also utilized. The SDSC is a standardized instrument with 26 questions and responses that are based on a 5-point Likert scale ("never," "occasionally," "sometimes," "often," and "always"). The total score varies from 26 to 130 and is distributed across six

subscales. The cutoff point for each subscale is indicated between the parentheses: difficulty initiating and maintaining sleep (DIMS – score ≥ 22); sleep-disordered breathing (SDB; score > 7); disorders of arousal (DA; score > 12); sleep-wake transition disorders (SWTD; score > 24); disorders of excessive sleepiness (DOES; score > 20); and sleep hyperhidrosis (SHY; score > 8). The scores of the subscales are inversely correlated with the quality of sleep. Scoring above the cutoff in at least one subscale indicates the presence of a sleep disorder. The total score and subscale scores were converted to T-scores; a T-score >70 (two standard deviations above the mean) was considered to be pathological.

2.4. Assessment of endothelial function

A protocol established for the ultrasonographic evaluation of the brachial artery was used to assess endothelial function [22,23]. The exams were performed noninvasively in the Cardiovascular Research Laboratory of the Bahiana School of Medicine and Public Health (EBMSP). The equipment used was a VIVID three ultrasound system (General Electric Company – Israel) with a multifrequency ultrasonic transducer ranging from 7 to 12 MHz. Endothelium-dependent vasomotor function was assessed by flow-mediated dilation measured, with reactive hyperemia. All examinations were conducted by the same investigator who had completed specific training for the exam and was blinded to the patient's diagnosis. The exams were performed in a calm environment at 22 °C after the patient had fasted for at least four hours, and rested quietly for 30 min. To control for circadian variations, all of the exams were performed in the morning. The patients were examined in a supine position with the left arm positioned ergonomically. The electrocardiogram was synchronized, and the heart rate was checked. The mercury column sphygmomanometer cuff functioned as a pneumatic tourniquet around the right forearm below the elbow flexure. The diameter of the brachial artery was measured in a longitudinal section with a high-resolution vascular ultrasound instrument and then was identified in light at the anterior wall media-adventitial interface ("M line") and the posterior wall intima-lumen interface. The Doppler sample was positioned at a 60° angle with grayscale control adequacy, depth, filter, and Doppler scale. The flow increase was induced by cuff insufflation around the arm to 250 mmHg for 4 min with continuous monitoring of the artery image, followed by cuff disinflation leading to reactive hyperemia. The first five flows (Doppler maximum velocity measurement) and artery diameter were monitored for 120 s (to measure the diameter at the end of 60 s). The FMD was then calculated as the percent change in diameter compared to the baseline resting diameters.

2.5. Assessment of laboratory data

After the endothelial dysfunction test, laboratory data were collected after an eight hour fasting period; laboratory data included the measurement of hemoglobin, leukocytes, reticulocytes, and LDH and the high-sensitivity C-reactive Protein (HS-CRP) by the turbidimetry method, all analyzed by a trustworthy laboratory. Levels of IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-17A and TNF-α were measured in sickle cell anemia patients in a steady state and healthy controls. Samples of plasma were purified and stored frozen at −80 °C before the batch-wise Luminex assays (Bio-Rad, Hercules, CA).

2.6. Statistical analysis

For the descriptive analysis, the quantitative variables are presented as the mean and standard deviation (SD) if their distributions were normal and as medians and interquartile ranges (IQR) if they were non-normal. Categorical variables are presented as simple frequencies and percentages. The Kolmogorov–Smirnov and Shapiro–Wilk normality tests were applied to evaluate the distribution of the variables, Bivariate and correlation analyses were performed to compare the categorical variables, and either the chi-square test or Fisher's exact test were used when necessary through bivariate analysis. To compare the means, we used the Student's t-test for independent samples and the Mann–Whitney U test to compare the medians of numeric variables with non-parametric distributions. To compare the means of the numeric variables with more than two categorized groups, we used ANOVA with the Bonferroni or Kruskal–Wallis corrections for ranking comparisons if the distribution was non-parametric. We used a two-tailed Spearman's correlation analysis to explore the linear relationship between SDSC scores and cytokines values. The software used for the database and statistical analysis was the Statistical Package for the Social Sciences for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA).

3. Results

The full sample comprised 122 individuals, with 60 in the HbSS group and 62 healthy children and adolescents in the comparison group; their average ages ±SD were 12.6 (3.1) and 11.6 (3) years, respectively, $p = 0.10$. The demographic characteristics of the participants with and without SCA are described in Table 1.

Regarding the SDSC variables, the total score resulting from the addition of the subscale scores later converted to T-scores >70, and the individual subscale scores were considered for analysis. Fourteen (24.6%) individuals in the case group exhibited symptoms of sleep disorders. The SDSC scores of the participants with or without SCA are described in Table 2. Comparisons of the correlated mean (±SD) and median (IQR) scores on the SDSC and the ESS between the children with SCA who were either using or not using hydroxyurea showed that the children with HbSS who were using hydroxyurea had higher scores on the SDB, DOES and SHY than the group without hydroxyurea [mean (±SD) 7.4 (3.9) *vs* 5.9 (2.9), $p = 0.026$; 7.5 (3.5) vs 6.9 (3.0), $p = 0.028$; 4.3 ± 3.2 vs 3.9 ± 2.6, $p = 0.044$, respectively]. These and the other comparisons are described in Table 3.

The median (IQR) value of the percent flow-mediated dilation (FMD) was lower in HbSS group than in the comparison group: 10.1 (6.4; 14) *vs*. 16.9 (12; 26.4), respectively ($p = 0.001$). When comparing the median (IQR) value between the HBSS groups of users and non-users of hydroxyurea, the FMD was similar ($p = 0.56$). There were no statistically significant differences in the SDSC and ESS scores between the participants with and without ED according to the percent FMD of the children with HbSS, although the mean values found in those with ED were higher than in those without ED. Subscale comparisons are shown in Table 4.

By analyzing the levels of inflammatory cytokines and HS-CRP in the total population, comparing patients with and without sleep disorders, we found no statistically significant difference. When analyzing patients with sickle cell anemia according to the presence or absence of sleep disorders, we also did not observe a statistically significant difference. However, we found a weak and positive correlation between DIMS and IL-12p70 ($r_s = 0.3$, $p = 0.03$) and IL1β ($r_s = 0.4$, $p = 0.01$); between DOES and TNF-α ($r_s = 0.3$, $p = 0.02$), IL6 ($r_s = 0.3$, $p = 0.03$), IL10 ($r_s = 0.3$, $p = 0.046$), e IL17A ($r_s = 0.3$, $p = 0.04$), ESS and HS-PCR ($r_s = -0,3$, $p = 0.05$) and finally between DA with IL6 ($r_s = 0.3$, $p = 0.04$). These and the other correlations are shown in Table 5.

Table 1
Demographic, clinical and laboratory characteristics of children with or without sickle cell anemia.

Variables	Children with sickle cell anemia (n = 60)	Children without sickle cell anemia (n = 62)	p value
Age (years)[a]			
Range	10–15	9–14	
Mean ± SD	12.6 ± 3.1	11.6 ± 3	0.10
Median (IQR)	13.5 (10; 15)	11 (9; 14)	
Gender (n°/%)[b]			
Male	38/63.3%	25/40.3%	**0.01**
Self-reported ethnicity[b]			
White (n°/%)	3 (5.1%)	8 (13.1%)	0.13
Non-white (n°/%)	56 (94.9%)	53 (86.9%)	
BMI (Kg/m^2)[a]			
Mean ± SD	16.6 ± 2.1	18.6 ± 3.7	**0.002**
BMI z score[a]			
Mean ± SD	−1.1 ± 0.9	−0.1 ± 1.4	
Median (IQR)	−1 (−1.5; −0.6)	−0.01 (−1.3; 1.1)	**0.002**
Use of hydroxyurea − n/%	26 (44.1)		
ACT (episodes ≥ 2/past year) − n/%	3 (5.1)		
VOC (episodes ≥ 3/past year) − n/%	17 (28.9)		
Pneumonia (episodes ≥ 2/past year) − n/%	17 (14.5)		
LDH (mean ± SD)	1173 UI/L ± 582		
Leukocyte count (mean ± SD)	12.186/mL ± 3778		
Reticulocyte count (mean ± SD)	7.9% ± 4.9		
Mean Hb (mean ± SD)	8 mg/dL ± 0.9		
SpO_2 (mean% ± SD)	93.9% ± 3.5		

SD: standard deviation; **IQR**: interquartile range; **BMI:** body mass index; **ACT:** acute chest syndrome; **VOC:** vaso-occlusive crisis; **LDH**: lactate dehydrogenase; **Hb:** hemoglobin; **SpO_2:** oxyhemoglobin peripheral saturation measured with a pulse oximeter.
[a] *Student's t*-test.
[b] χ^2 test.

Table 2
Scores on the Sleep Disturbance Scale for Children (SDSC) and Epworth Sleepiness Scale (ESS) of children with or without sickle cell anemia (SCA).

Variables	Total sample (n = 118)	With SCA (n = 57)	Without SCA (n = 61)	p value[a]
Global score (SDSC)				
Mean ± SD	45.1 ± 12.1	44.7 ± 11.5	45.6 ± 12.7	0.76
Median (IQR)	42 (36.8; 52)	41 (37; 50.5)	42 (36.5; 53.5)	
DIMS				
Mean ± SD	12.4 ± 4.3	12.1 ± 4	12.7 ± 4.5	0.49
Median (IQR)	11 (9; 15.5)	11 (9; 14)	12 (9; 15.5)	
SBD				
Mean ± SD	5.9 ± 3.1	6.7 ± 3.4	5.2 ± 2.7	**0.04**
Median (IQR)	5 (3; 7)	7 (4; 8)	5 (3; 7)	
DA				
Mean ± SD	4.1 ± 1.7	4.1 ± 1.6	4.1 ± 1.9	0.97
Median (IQR)	3 (3; 5)	3 (3; 5)	3 (3; 5)	
SWTD				
Mean ± SD	11 ± 4.6	11 ± 4.9	11.1 ± 4.3	0.53
Median (IQR)	10 (7; 14)	10 (6.5; 14)	11 (7; 14.)	
DOES				
Mean ± SD	8.1 ± 3.7	7.1 ± 3.2	9.1 ± 3.8	0.02
Median (IQR)	7 (5; 10)	6 (5; 8.5)	9 (5; 12)	
SHY				
Mean ± SD	3.6 ± 2.7	4 ± 2.7	3.3 ± 2.7	0.06
Median (IQR)	2 (2; 5)	2 (2; 6)	2 (2; 3)	
EES				
Mean ± SD	6.6 ± 4.4	6.6 ± 4.6	6.6 ± 4.2	0.74
Median (IQR)	6 (3; 9)	6 (3; 9)	6 (3; 9)	

DIMS: disorders of initiating and maintaining sleep; **SBD**: sleep breathing disorders; **DA**: disorders of arousal; **SWTD**: sleep-wake transition disorders; **DOES**: disorders of excessive sleepiness; **SHY**: sleep hyperhidrosis; **ESS**: Epworth Sleepiness Scale; **SD:** standard deviation; **IQR:** interquartile range.
[a] ANOVA test.

4. Discussion

The results of the present study show that the children with SCA exhibited higher sleep-disordered breathing scores than the healthy participants in the total sample. Our results correspond with those described in the literature [16,17]. Sleep-disordered breathing comprises a spectrum of disorders, including OSA, which are associated with increases in upper airway resistance during sleep, and it affects up to 13% of prepubertal children [27]. Similar to our findings, Daniel et al., in 2010, used the Children's Sleep Habits Questionnaire to analyze 54 children with sickle cell disease compared to controls and found that the case group exhibited more episodes of night waking, enuresis, and SDB, which resulted in nocturnal hypoxemia, excessive daytime sleepiness, and the consequent impairment of daily living activities [28]. Furthermore, Salles et al., assessed 85 children with SCA aged 2–18 years using polysomnography; they detected snoring in 44.7%, and OSA in 10.6% [29] of patients. Rosen et al., evaluated 243 children

Table 3
Comparison of scores on the Sleep Disturbance Scale for Children (SDSC) and Epworth Sleepiness Scales (ESS) between patients with SCA who were either using or not using hydroxyurea.

Variables Mean ± SD Median (IQR)	SCA with hydroxyurea (n = 26)	SCA without hydroxyurea (n = 34)	p value[a]
DIMS	11.8 ± 3.8 11 (9; 14.5)	11.9 ± 4.0 11 (10; 13)	0.250
SDB	7.4 ± 3.9 7 (4.3; 10.5)	5.9 ± 2.9 5 (3; 7)	**0.026**
DA	4.2 ± 1.7 3 (3; 5)	4.0 ± 1.5 3 (3; 4.8)	0.940
SWTD	10.5 ± 3.9 10 (7.3; 13.5)	11.1 ± 5.4 10.5 (6; 13.8)	0.945
DOES	7.5 ± 3.5 7 (5; 8.8)	6.9 ± 3.0 5.5 (5; 8.8)	**0.028**
SHY	4.3 ± 3.2 2.5 (2; 6)	3.9 ± 2.6 2 (2; 5.8)	**0.044**
EES	5.9 ± 4.4 6 (3; 7)	7.0 ± 4.7 6 (3; 11.8)	0.617

DIMS: difficulty initiating and maintaining sleep; **SDB:** sleep-disordered breathing; **DA:** disorders of arousal; **SWTD:** sleep-wake transition disorders; **DOES:** disorders of excessive sleepiness; **SHY:** sleep hyperhidrosis; **ESS:** Epworth Sleepiness Scale.
[a] Mann-Whitney test.

Table 4
Comparison of scores on the Sleep Disturbance Scale for Children (SDSC) and Epworth Sleepiness Scales (ESS) between patients with and without endothelial dysfunction according to the percent flow-mediated dilation of children with sickle cell anemia.

Variables	FMD < 8.4% (n = 19)	FMD > 8.4% (n = 32)	p value[a]
DIMS			
mean ± SD	13.2 ± 4.5	11.6 ± 3.9	0.61
median (IQR)	11.5 (10; 16.3)	11 (8.8; 14)	
SDB			
mean ± SD	7.8 ± 4.3	6.5 ± 2.6	0.15
median (IQR)	7 (3.8; 11.3)	7 (5; 7.3)	
DA			
Mean ± SD	4.3 ± 2.0	4.1 ± 1.4	0.28
median (IQR)	3 (3; 7)	3.5 (3; 5)	
SWTD			
mean ± SD	12.2 ± 5.5	10.3 ± 4.8	0.41
median (IQR)	11.5 (7.8; 14.3)	9.5 (6; 13)	
DOES			
mean ± SD	9.1 ± 4.4	6.4 ± 2.3	0.26
median (IQR)	8 (5; 12.3)	5 (5; 8)	
SHY			
mean ± SD	4.8 ± 3.5	3.6 ± 2.1	0.7
median (IQR)	2 (2; 9.3)	2.5 (2; 5.3)	
EES			
mean ± SD	7.6 ± 5.4	6.4 ± 4.5	0.22
median (IQR)	6.5 (2.8; 12.3)	5.5 (3; 7.5)	
SRD			
mean ± SD	50.9 ± 12.9	42.4 ± 9.9	0.1
median (IQR)	50 (42.8; 55.5)	40 (36; 45)	

SDSC: Sleep Disturbance Scale for Children; DIMS: disorders of initiating and maintaining sleep; SBD: sleep breathing disorders; DA: disorders of arousal; SWTD: sleep-wake transition disorders; DOES: disorders of excessive sleepiness; SHY: sleep hyperhidrosis; ESS: Epworth Sleepiness Scale; SD: standard deviation; IQR: interquartile range; SRD: sleep-related disorders.
[a] Mann-Whitney test.

(median age of 10 years) using a questionnaire and polysomnography; they concluded that children with SCA have a higher prevalence of sleep-disordered breathing consistent with OSAS, beyond greater nocturnal desaturation, and that these children experience typical nocturnal symptoms of snoring and breathing/sleep disturbances [30].

We assessed endothelial function using flow-mediated vasodilation, measured using reactive hyperemia; SCA patients exhibited

Table 5
Correlations amongst inflammatory cytokines, high sensitivity CRP, and scores on the Sleep Disturbance Scale for Children (SDSC) and Epworth Sleepiness Scale (EES) of children with sickle cell anemia.

Variables	IL1β	IL6	IL8	IL10	IL12p70	IL17A	TNF-α	HS-PCR
DIMS[a]	**0,38**†	0,19	−0,12	0,16	0,32†	0,29	0,13	−0,12
SDB[a]	0,001	0,05	−0,07	−0,19	−0,11	−0,17	−0,004	−0,09
DA[a]	0,14	**0,3**†	0,03	0,17	0,19	0,27	0,04	−0,002
SWTD[a]	0,07	0,17	0,21	−0,04	−0,12	0,08	0,20	−0,03
DOES[a]	0,08	**0,32**†	0,11	**0,29**†	0,07	**0,30**†	**0,30**†	0,006
SHY[a]	−0,13	0,09	0,26	−0,16	−0,06	0,11	0,12	0,14
ESS[a]	−0,04	0,01	−0,13	−0,002	0,14	0,08	0,20	**−0,29**†

DIMS: difficulty initiating and maintaining sleep; **SDB:** sleep-disordered breathing; **DA:** disorders of arousal; **SWTD:** sleep-wake transition disorders; **DOES:** disorders of excessive sleepiness; **SHY:** and sleep hyperhidrosis; **ESS:** Epworth Sleepiness Scale.
† $p \leq 0,05$.
[a] Spearman's correlation.

on average lower FMD values than those of healthy individuals. Gozal et al., conducted a polysomnography study that assessed endothelial function by a modified hyperemic test after cuff-induced occlusion of the brachial artery and measured the levels of the endothelial activation marker sCD40L in 26 non-obese children with OSA and eight matched, healthy children. They observed a blunted post-occlusive hyperemic response and high sCD40L levels in the snoring children with OSA, which improved after adenotonsillectomy in the majority [31] of case subjects. Interactions between activated endothelium and sickle cell erythrocytes might contribute to the vascular complications of sickle cell disease and thus endothelial dysfunction in these children.

Using the 10th percentile as a cutoff value for endothelial dysfunction in healthy children, according to references by Järvisalo et al., [32,33] the value of FMD was found to be 8.4%. In the present study, we compared the median (IQR) values of FMD and found that these values were substantially lower among children in the SCA group compared with the healthy, age-matched children. This finding is pertinent because we hypothesized that vascular aggression in SCA might occur during the early stages, although this specific question has not been directly tested by our group. De Montalembert et al., [15] analyzed the endothelial function of 21 children with SCA (who were asymptomatic at the time of enrollment, had no blood transfusion in the previous three months, and who were not being treated with hydroxyurea), to those of 23 controls, and observed significantly lower endothelial dilation values in the case group, with a mean ± SD FMD of 5.6 ± 0.2 versus 8.0 ± 0.2, respectively. Hadeed et al., studying children and adolescents with sickle cell disease, did not demonstrate any significant alteration of endothelial function or significant change in arterial wall properties in children with SCD [34]. However, Von der Land et al., studying children and adolescents with sickle cell disease, with the genotype HbSS, HbSβ°, HbSC or HbSβ+, demonstrated that even young children in a stable clinical condition show signs of persistent endothelial dysfunction [35]. In this present study, FMD was significantly lower in children and adolescents with stable SCA than in healthy subjects, suggesting a smaller capacity of vasodilation due to the impairment of endothelial function in SCA. In a study recently published by us, the ROC curve analysis demonstrated that FMD values could distinguish acute chest syndrome (ACS) from non-ACS patients with an accuracy of 71% and specificity of up to 79% [36]. Our patients were matched by age. However there was an increased frequency of female individuals in the group of healthy controls compared to that in the SCA group. We did not find differences in FMD between males and females. Previous reports have indicated that there is an age-related decline in FMD values and that healthy male

individuals display lower FMD values than age-matched females [23]. However, in a study recently published by our group, we found that there were no differences in FMD values between male and female study participants within a given study group and that FMD values were found to be reduced in SCA patients irrespective of gender [36].

Our patients with SCA and ED exhibited higher SDSC scores than patients without ED; however, these differences were not significant. There are similarities and confluences of the various pathogenic pathways in SCD and sleep-disordered breathing, so it seems legitimate to formulate hypotheses that sleep-disordered breathing exacerbates the pathophysiological pathways underlying the manifestations of SCD [19]. Brunetti et al., investigated 23 children with sleep-disordered breathing and 32 controls using night polysomnography and ED assessment; FMD was measured via brachial artery ultrasound. These authors observed a negative correlation between FMD and the apnea-hypopnea index (AHI) and concluded that OSA might impair endothelial function and worsen cardiovascular risk profile during childhood [37–39]. Previous data show that data from questionnaires as well as parent reporting of symptoms are inaccurate measures of the presence and severity of OSA; we recommend that objective tests such as polysomnography be used in the diagnosis of OSAS. This recommendation becomes even more important in children with SCA in whom other disorders of oxygen supply, such as anemia, oxyhemoglobin desaturation, or other pulmonary dysfunctions, are often present [30].

Analysis of the scores on the sleep disorder subscales comparing the children who used hydroxyurea and the ones who did not shows that the former exhibited higher sleep-disordered breathing scores than the latter. Mousinho-Ribeiro et al., reported in 2008 that low fetal hemoglobin (HbF) levels were associated with poorer clinical progression of patients with SCA and worsening of hemolysis and VOC; hydroxyurea increased plasma HbF levels, thus inducing clinical improvement in these patients [40]. On these grounds, the higher SDSC scores exhibited by the patients using hydroxyurea in the present study might be explained by the fact that they were in poorer clinical condition and thus exhibited more sleep-related disorders. Narang et al., assessed 37 hydroxyurea -using children with SCA who were matched to 104 healthy children who were not using hydroxyurea and concluded that despite the increase in awake and nocturnal SpO_2 in the former, there were no significant differences in the frequency of OSA or the severity of the sleep AHI between the groups [41]. The impact of hydroxyurea on sleep disorders is not yet well documented in the literature; more studies are needed to determine whether this drug improves or worsens sleep-related breathing symptoms [41–43]. As we did not have any information on our patients before starting hydroxyurea, the occurrence of sleep-disordered breathing might not have been influenced by this medication. However, this study was not designed to evaluate whether hydroxyurea treatment influences the risk of disordered sleep breathing.

This study failed to demonstrate differences between cytokines and HS-CRP levels in the total population between children with and without sleep disorders, although it has been demonstrated in SCA children a significant and positive correlation of some cytokines and TNF-alpha with DIMS, DA, DOES, and ESS. Graido-Gonzalez et al., evaluating the levels of interleukins (IL) and TNF-α in HbSS individuals during and after vaso-occlusive crises, did not observe an increase in these markers in any of these situations. Also, no difference was observed in levels of IL-1β, IL-6, IL-8, IL-10, and TNF-α between the sickle and healthy afro-descendant controls [44]; however, in this study, it is noteworthy that several pro-inflammatory cytokines were related to markers of sleep disorders in patients with SCA. Although no simple and useful disease marker panel for OSA is currently available to be routinely used in clinical practice, studies in adults have explored mainly the investigation of IL-6, TNF-α and HS-CRP as potentially promising biomarkers; however, no specific biomarker has been tested by most authors of pediatric studies [45]. Inflammatory cytokines such as TNF-a, IL-6, and IL-17 play an important role in the inflammatory response in SCA. Studies indicate that TNF-α is the main mediator of the acute inflammatory response, recruiting neutrophils and monocytes to the site of inflammation [6]. It is possible that the sample size was not sufficient to demonstrate associations between SDSC scores and cytokines; further study is needed to define the relative contribution of each cytokine in sleep disorders.

The present study was limited by its cross-sectional design and the use of retrospective data collected from medical records, which restricted the scope of the study and the generalization of the results. Another limitation was the use of subjective reports by the parents of the children through the use of questionnaires; unfortunately, no objective measure of sleep, such as polysomnography, was included, which might have corroborated the data in these reports. There is no consensus about which FMD value should be used to assume increased or reduced vascular damage in children and adolescents with SCA, which makes it essential to compare them to healthy control groups. The study design can not explain clearly the association between the elevation of proinflammatory interleukins in children with SCA and sleep-related disorders. It is likely that different techniques used to quantify the cytokines, sample size, chosen statistical analysis, and disease polymorphism may have influenced these results.

5. Conclusion

This study has shown that children with SCA presented higher scores on the SDSC and ESS scales when compared to matched healthy children; collected data indicated that sleep-disordered breathing is more prevalent in patients with SCD. We observed that FMD values were significantly lower in children and adolescents with stable SCA than in healthy subjects, and the patients with SCA and lower FMD values exhibited higher sleep-disordered breathing scores. In SCA children a significant and positive correlation of some cytokines and TNF-alpha with DIMS, DA, and DOES was found. Future studies assessing sleep in children with SCA, especially those using hydroxyurea, using polysomnography are needed, correlated with new methods for investigating ED, such as the measurement of circulating exosomal microRNAs [46].

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Conflicts of interest

The authors declare no conflict of interest.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2018.08.019.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2018.08.019.

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