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Sleep Disorders in Adult Sickle Cell Patients

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Study Objectives: While sleep apnea has been studied in children with sickle cell disease (SCD), little is known about sleep disorders in adult sickle cell patients. The objective of this study was to evaluate sleep disordered breathing and its polysomnographic characteristics in adult patients with sickle cell disease.

Methods: The analysis cohort included 32 consecutive adult SCD patients who underwent a comprehensive sleep evaluation and overnight polysomnography in an accredited sleep center after reporting symptoms suggesting disordered sleep or an Epworth Sleepiness Scale score ≥ 10. Epworth score, sleep parameters, comorbid conditions, and narcotic use were reviewed and compared in patients with and without sleep disordered breathing. SCD complication rates in the two groups also were compared.

Results: In adult SCD patients who underwent overnight polysomnography, we report a high prevalence (44%) of sleep disordered breathing. Disease severity was mild to moderate (mean apnea-hypopnea index = 17/h (95% CI: 10–24/h). Concomitant sleep disorders, including insomnia complaints (57%) and delayed sleep-phase syndrome (57%), also were common in this population. In this limited cohort, we did not find increased SCD complications associated with sleep disordered breathing in adult patients with sickle cell disease.

Conclusions: A high burden of sleep disordered breathing and other sleep-related complaints were identified in the adult sickle cell population. Our results provide important information on this unique population.

Keywords: sleep, obstructive sleep apnea, anemia, sickle cell

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Sickle cell disease (SCD) is a common genetic disorder of hemoglobin with complications that have a significant impact on the quality of life of affected individuals.[1] Sleep disordered breathing (SDB) is a group of conditions characterized by complete or partial cessation of breathing while sleeping. Obstructive sleep apnea (OSA), the most prevalent form of SDB, is a disorder that affects approximately 2% to 4% of the adult population.[2,3] It is defined by daytime sleepiness, as well as 5 or more obstructive respiratory events per hour of sleep (witnessed apneic spells and awakenings secondary to gasping or choking).[4]

Obstructive sleep apnea and its polysomnographic (PSG) characteristics have been studied in children with SCD.[5–10] Children with SCD referred to sleep laboratory have a high prevalence (69%) of OSA.[8] However, an increasing percentage of SCD patients live into adulthood.[11] Since most studies on OSA and SCD have been conducted in children, extrapolating these data to apply to the adult sickle cell population may not be appropriate. Using a validated palliative care tool, a survey of adult patients at our sleep center identified pain and sleep disturbance as two key symptoms of adult SCD.[12] Consequently, we examined the reasons for sleep problems in this patient population that may affect quality of life and impact the severity of complications. A recently published study on sleep in adult sickle cell similarly revealed that more than 70% of adult patients with sickle cell disease have sleep disturbances; however, PSG data were not obtained.[13]

Defining and studying sleep disorders in adult SCD patients are important, as management of adult SDB is quite different from pediatric patients (e.g., positive airway pressure therapy versus tonsillectomy and adenoidectomy). The majority of adult sickle cell patients in our cohort are on chronic narcotics, and the possibility of central sleep apnea also needs to be considered. The purpose of this study was to describe the PSG characteristics of adult SCD and to compare baseline characteristics and PSG findings in patients with SDB versus those without SDB. We also investigated whether patients with SDB have more sickle cell complications than those without SDB.

BRIEF SUMMARY

Current Knowledge/Study Rationale: High prevalence of sleep apnea has been reported in children with sickle cell disease, however, data on adult sickle cell patients is lacking. With increasing number of sickle cell patients reaching adulthood, we investigated prevalence and characteristics of sleep disorders in adult sickle cell patients.

Study Impact: The study adds to our existing knowledge and suggests high prevalence of sleep disorders in adult sickle cell patients. The study also suggests that Epworth Sleepiness Scale and overnight pulse-oximetry may be cost effective screening tools for detecting sleep disordered breathing in this population.

METHODS

Study Design

This was an analysis of a prospectively maintained database of adult patients with SCD who were referred to and evaluated at the sleep disorders center at East Carolina University (ECU) from January 2009 to April 2011. Those evaluated either reported problems with sleep during their routine adult sickle cell clinic visits or had a score ≥ 10 on the Epworth Sleepiness Scale (ESS) administered at the clinic. All patients underwent an interview including a sleep-focused physical examination by a board certified sleep physician before PSG was conducted. Following approval by the institutional review board, demographic, clinical, and PSG data were collected from the ECU electronic health records. Individuals aged ≥ 18 years with homozygous hemoglobin SS disease were included in the study (confirmed by hemoglobin electrophoresis).

Polysomnography consisted of an electrocardiograph, electroencephalograph, continuous oronasal airflow recording with both thermistor and pressure transducer, recording of chest wall and abdomen movement using respiratory inductive plethysmography belts, pulse oximetry, and chin electromyography. All sleep studies were performed at a university sleep disorders center accredited by the American Academy of Sleep Medicine (AASM), using the Cadwell Easy III PSG system (Kennewick, WA). Sleep was staged by a registered PSG technologist according to AASM scoring guidelines (AASM scoring manual).[14] Hypopneas were defined as 50% reduction in flow accompanied by 4% oxygen desaturation. Polysomnograms were interpreted by a single, board-certified sleep physician. Based on PSG results, the sample was classified into 2 groups: (a) SCD with SDB and (b) SCD without SDB.

Sleep disordered breathing was defined as an apnea-hypopnea index (AHI, events/h) ≥ 5. Mild SDB was defined as AHI of 5 to ≤ 15, moderate as AHI of 15 to < 30, and severe as ≥ 30. The diagnosis of central sleep apnea was made if the central apnea index (CAI) was ≥ 5 and if > 50% of the respiratory events were interpreted as central. Significant oxygen desaturation was defined as oxygen saturation < 89% for ≥ 5 cumulative minutes during the sleep study. The oxygen desaturation index (ODI) was defined as the number of recorded oxygen desaturations ≥ 4%/h of sleep. Insomnia complaints were defined as difficulty initiating sleep (sleep latency > 60 min) or difficulty maintaining sleep (> 2 awakenings requiring > 20 min to fall back asleep) on the majority of nights for > 4 weeks.

Delayed sleep phase syndrome (DSPS) was defined as delay in sleep onset ≥ 2 h from the desired sleep time and inability to awaken at desired time. The above pattern had to be persistent for ≥ 3 months. Patients were considered to have periodic limb movements (PLMS) if ≥ 15 events per hour were recorded. A mini-crisis was defined as a pain crisis at home requiring escalation of narcotics that did not result in an emergency room visit or hospitalization. To determine if crises occurred more commonly at night in this cohort because of sleep disorders, patients were asked to record the times of day when they experienced pain crises. The percentage of night crises was determined by the subjective report of the amount of time that the patient had experienced pain crisis during the night compared with the total amount of time of pain crises.

Statistical Analysis

Categorical variables were presented as frequency and percentage; continuous variables were presented as the mean (plus or minus 1 standard deviation). Statistical significance was tested using the two-sample t-test for continuous variables and the Fisher's exact test for categorical variables. Multivariable log-normal plots were used to assess normality and heteroscedasticity. When appropriate, a stabilizing transformation (e.g., logarithm or square root) was applied to the data. Statistical significance was defined as $p \leq 0.05$. SAS software (version 9.3) was used for all analyses.

RESULTS

A total of 50 consecutive patients were referred to the ECU sleep disorders center for evaluation of self-reported sleep problems or excessive daytime sleepiness based on the ESS score. Eighteen (36%) patients did not receive a sleep study and were lost to follow up. Thirty-two patients (64%) underwent a comprehensive sleep evaluation including PSG. Based on the PSG test results, 14 of these 32 patients (44%) were diagnosed with SDB (AHI ≥ 5). Mean age of the SDB group was 40 years (**Table 1**). Patients with SDB demonstrated increased REM latency (159 min vs. 98 min; p = 0.014), increased ODI (13 vs. 1.6; p = 0.0009), and increased AHI (17 vs. 1.6; p = 0.0001; **Table 2**). Baseline characteristics, which included comorbid conditions such as hypertension, asthma, chronic obstructive pulmonary disease, diabetes mellitus, stroke, avascular necrosis, ulcers, history of smoking, use of narcotics and hydroxyurea, pulmonary hypertension by tricuspid regurgitation jet, were not significantly different in the SDB group vs. the non-SDB group (**Table 3**). Although the mean ODI was less than the mean AHI, it was closely associated with the presence of SDB. Overnight PSG demonstrated predominantly central apnea (CAI = 27) in 1 of the 14 patients (7%). SDB was mild to severe with a mean AHI of 17 (mild SDB in 8 patients, moderate SDB in 4 patients, and severe SDB in 2 patients).

The SDB group had a mean body mass index (BMI) of 32.6 kg/m^2 vs. 24 kg/m^2 for the non-SDB group (p = 0.0035). Mean Epworth scores were 13.0 vs. 8.6 for the SDB and non-SDB groups, respectively (p = 0.017). The mean neck size in patients with SDB was 38.1 cm vs. 33.0 cm in non-SDB (p = 0.007). In addition, 21 (62%) patients with SCD had insomnia symptoms and 17 (53%) also demonstrated DSPS. Patients with SDB tended to have more daytime sleepiness and a trend towards nocturia (p = 0.063).

The average number of hospital admissions in the last 5 years for SCD patients with SDB was 9.14 in 5 years vs. 6 admissions in 5 years without SDB (p = 0.15). The percent of mini-crisis was 2.7 per month (95% CI = 1.3–4.1]) in SCD with SDB vs. 3.6 per month (95% CI = 1.4–5.7) in SCD without SDB (p = 0.69; **Table 4**).

Table 1—Demographic and sleep characteristics in SDB versus Non-SDB.

Characteristics	SDB (n = 14)	Non-SDB (n = 18)	p value*
Age, years, mean (95% CI)	41 (35–47)	38 (32–44)	0.48
Males, n	6	4	0.27
Body mass index, kg/m^2, mean (95% CI)	33 (27–38)	24 (22–27)	0.0035
Neck size, cm, mean (95% CI)	38.1 (35.5–40.6)	33 (33–35.5)	0.0070
Epworth Sleepiness Scale, mean (95% CI)	13 (10–15)	8.6 (6.2–11)	0.017
Apnea-hypopnea index, mean (95% CI)	17 (10–24)	1.6 (0.98–2.1)	< 0.0001
Oxygen desaturation index, mean (95% CI)	13 (6.4–19)	1.6 (1.0–2.1)	0.0009
Nocturia, mean (95% CI)	2.3 (1.6–3.0)	1.6 (1.0–2.1)	0.063
Insomnia complaints, n (%)	8 (57)	13 (72)	0.46
Delayed Sleep Phase Syndrome, n (%)	8 (57)	9 (50)	0.73

*p values were computed using an exact two-sample T-test for continuous variables and a Fisher exact test for categorical variables. SDB, sleep disordered breathing.

Table 2—Sleep architecture characteristics.

Characteristics	SDB (n = 14) Mean (95% CI)	Non-SDB (n = 18) Mean (95% CI)	p value*
Total sleep time, min	323 (281–366)	341 (312–370)	0.45
Sleep latency, min	25 (0.96–48)	12 (3.6–21)	0.062
Rapid eye movement latency, min	159 (111–208)	98 (52–143)	0.014
% Stage N1	5.9 (3.7–8.2)	8.8 (4.2–13)	0.25
% Stage N2	71 (64–79)	65 (57–72)	0.21
% Stage N3	8.5 (−0.47–17)	10 (2.1–19)	0.74
Arousal index	16 (6.8–25)	13 (9.2–18)	0.63
Oxygen saturation nadir	82 (79–85)	83 (79–86)	0.79

*p values were computed using an exact two-sample T-test. SDB, sleep disordered breathing.

DISCUSSION

Our study reveals a high prevalence of SDB (44%) in an adult population of SCD patients with symptoms of disturbed sleep or excessive daytime sleepiness (ESS > 10) referred to an accredited sleep disorders center. Similarly, Wallen and colleagues surveyed 388 adults in a research cohort using the Pittsburgh Sleep Quality Index and the Beck Depression Inventory, and found a high prevalence of sleep disturbances as well as correlation of sleep disturbances with depression.[14] Our study complements this survey-based study by adding PSG data. In addition, our findings document a high use of narcotics in this population.

In the past, a high prevalence of SDB has been observed in children with SCD referred for sleep symptoms.[8] Increased prevalence of OSA in children with SCD is thought to be due to excessive adenoid and tonsillar growth.[8,15] Since the mean age group in our cohort was 40 years (an age when there should be little tonsillar or adenoidal tissue), and no obvious tonsillar enlargement or oro-pharyngeal abnormalities were recorded on examination of the patients, it is unlikely to be the causative factor of SDB in adult SCD patients.

Increased neck size and higher BMI appear to be significant risk factors for SDB in adult sickle cell patients, as in the general population. However in our study, for the SCD with SDB group, the mean neck size was 38.1 cm, which is lower than described as a risk factor for the general population.[2] Nevertheless, the larger neck size and the higher BMI found in the SDB group reinforce the concept that excess adipose tissue is important in the pathogenesis of SDB even in SCD patients. As would be expected, the SDB group also demonstrated differences in sleep and REM latency. However, presence of SDB

Table 3—Comorbid condition in SCD/SDB versus SCD/Non-SDB.

Comorbid Conditions	SDB (n = 14) n (%)	Non-SDB (n = 18) n (%)
Hypertension	7 (50)	7 (39)
Asthma	2 (14)	4 (22)
Chronic obstructive pulmonary disease	0 (0)	1 (5)
Diabetes mellitus	1 (7)	0 (0)
Stroke	2 (14)	4 (22)
Avascular necrosis	1 (7)	5 (28)
Ulcers	1 (7)	4 (22)
Smoking	4 (28)	2 (11)
Narcotic usage	12 (86)	17 (94)
Hydroxyurea	7 (50)	11 (61)

SCD, sickle cell disease; SDB, sleep disordered breathing.

Table 4—Complication in SDB versus Non-SDB group.

Complications	SDB (n = 14) Mean (95% CI)	Non-SDB (n = 18) Mean (95% CI)	p value*
% of crisis during sleep	44 (22–65)	39 (24–53)	0.67
Average admissions last 5 years	9.1 (−0.73–19)	6.0 (3.7–8.3)	0.15
Average mini-crisis/month	2.7 (1.3–4.1)	3.6 (1.5–5.7)	0.69

*p values were computed using an exact two-sample T-test. SDB, sleep disordered breathing.

did not appear to have any impact on hospital admissions, mini-crisis or nighttime pain events as reviewed in the past 5 years of records. This may be attributed to the low number of patients or absence of severe SDB.

Although only one patient in our study was found to have non-obstructive sleep disordered breathing (central sleep apnea), this still may be a potential problem in SCD patients that merits attention by sleep medicine physicians if documented in future studies.[16] We speculate that the prevalence of opioid-induced sleep disorders may increase since patients with SCD are living longer and are exposed to extended use of narcotics. Over 90% of the patients in our study were on chronic narcotics for pain. Chronic use of opioids has been shown to be a risk factor for central sleep apnea.[17,18]

The mechanism of opioid induced central sleep apnea is postulated to be due to inhibition of inspiratory rhythm generators neurons located in the pre-Botzinger area.[19] We suggest that in this cohort of patients, end-tidal carbon dioxide monitoring or measurement of arterial blood gases should be considered.

In addition, our cohort of patients showed an increased burden of other sleep disorders including DSPS and insomnia complaints. Both DSPS and insomnia were equally represented in both the SDB and the non-SDB groups. The mean Epworth score was significantly higher in the SDB group despite the presence of other sleep disorders, suggesting that SDB produced excessive daytime sleepiness over what can be explained by DSPS and insomnia complaints. Compared with population-based historic data, the burden of insomnia in SCD patients is high.[20] Insomnia may be due to a high rate of depression reported in SCD patients (25% to 44%), pain issues, or other comorbid conditions.[21,22]

In contrast to significant PLMS observed in reports of children with SCD,[8] our study did not reveal any PLMS. We speculate that absence of PLMS in adult population may be due to high iron load in adult sickle cell patients. However data on ferritin levels were not available, as this information was not part of the study or clinical protocol.

The ODI was significantly increased in SCD patients (mean ODI: 13) with SDB and was related to the AHI (mean AHI: 17), suggesting that it may be a relatively simple and inexpensive method to screen for SDB in this population. Our prior study, utilizing pulse oximetry in adult patients with SCD, revealed that 45% of adult sickle cell patients had oxygen desaturations during sleep. A significant number had a high ODI, suggesting SDB as an important reason for oxygen desaturations.[23] Studies in the general population also have shown significant correlation between ODI and AHI.[24]

Limitations

A major limitation of this study is that only patients who had symptoms of disturbed sleep or an increased ESS were included. This limits the generalizability of our findings. Additionally, the sample size for this study was relatively small and consequently some comparisons lacked power to detect a statistically significant result. However, due to this unique patient population, most studies in the past also have had small sample sizes. A significant number of patients (36%) did not receive a sleep study despite efforts to facilitate the evaluations. However, the demographic characteristics of this group were similar to those who underwent a sleep study and it is not likely that their exclusion significantly biased our results. Future studies may be strengthened by the addition of a control group and a quality of life survey.

CONCLUSIONS

We identified a high burden of SDB, including OSA, and other sleep-related complaints in the adult sickle cell population screened by the ESS. We suggest that this simple tool be utilized in adult sickle cell clinics for screening sleep disorders. Overall, the risk factors for SDB among adult SCD patients in the current study were similar to that of the general population, with the exception of a smaller neck size. The possibility of central sleep apnea caused by the chronic use of narcotics in this population may warrant consideration in future studies.

There also appears to be a high burden of insomnia complaints and DSPS in patients with SCD. The ODI may be considered as a low-cost screening tool for SDB in this population. SDB does not seem to have an impact on SCD complications as measured by frequency of pain crisis and hospitalization in this limited number of patients. Given the paucity of data related to sleep disorders in adults with SCD, the current study provides important new information on this topic.

ABBREVIATIONS

AASM, American Academy of Sleep Medicine
AHI, apnea-hypopnea index
BMI, body mass index
CI, confidence interval
COPD, chronic obstructive pulmonary disease
DSPS, delayed sleep-phase syndrome
ECU, East Carolina University
ODI, oxygen desaturation index
OSA, obstructive sleep apnea

PLMS, periodic limb movements
PSG, polysomnography
REM, rapid eye movement
SCD, sickle cell disease
SDB, sleep disordered breathing

REFERENCES

1. Ashley-Koch A, Yang Q, Olney RS. Sickle hemoglobin (HbS) allele and sickle cell disease: a HuGE review. Am J Epidemiol 2000;151:839–45.
2. Epstein LJ, Kristo D, Strollo PJ, et al. Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults. J Clin Sleep Med 2009;5:263–76.
3. American Lung Association. Obstructive sleep apnea or sleep-disordered breathing. In: State of Lung Disease in Diverse Communities 2010. Washington DC: American Lung Association, 2010:69–72.
4. Duchna HW. Sleep-related breathing disorders--a second edition of the International Classification of Sleep Disorders (ICSD-2) of the American Academy of Sleep Medicine (AASM). Pneumologie 2006;60:568–75.
5. Ambrusko SJ, Gunawardena S, Sakara A, et al. Elevation of tricuspid regurgitant jet velocity, a marker for pulmonary hypertension in children with sickle cell disease. Pediatr Blood Cancer 2006;47:907–13.
6. Mullin JE, Cooper BP, Kirkham FJ, et al. Stability of polysomnography for one year and longer in children with sickle cell disease. J Clin Sleep Med 2012;8:535–9.
7. Needleman JP, Franco ME, Varlotta L, et al. Mechanisms of nocturnal oxyhemoglobin desaturation in children and adolescents with sickle cell disease. Pediatr Pulmonol 1999;28:418–22.
8. Rogers VE, Lewin DS, Winnie GB, Geiger-Brown J. Polysomnographic characteristics of a referred sample of children with sickle cell disease. J Clin Sleep Med 2010;6:374–81.
9. Salles C, Ramos RT, Daltro C, Barral A, Marinho JM, Matos MA. Prevalence of obstructive sleep apnea in children and adolescents with sickle cell anemia. J Bras Pneumol 2009;35:1075–83.
10. Spivey JF, Uong EC, Strunk RC, Boslaugh SE, DeBaun MR. Low daytime pulse oximetry reading is associated with nocturnal desaturation and obstructive sleep apnea in children with sickle cell anemia. Pediatr Blood Cancer 2008;50:359–62.
11. Platt OS, Brambilla DJ, Rosse WF et al. Mortality in sickle cell disease--life expectancy and risk factors for early death. N Engl J Med 1994;330:1639–44.
12. Lopez G, Liles DK, Knupp CL. ESAS (Edmonton Symptom Assessment System) as an effective outpatients serial-longitudinal symptom assessment tool in patients with sickle cell disease. Blood 2010;116:1553a.
13. Wallen GR, Minniti CP, Krumlauf M, et al. Sleep disturbance, depression and pain in adults with sickle cell disease. BMC Psychiatry 2014;14:207.
14. Iber C, Ancoli-Israel S, Chesson A, Quan S, for the American Academy of Sleep Medicine. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications. Westchester, IL: American Academy of Sleep Medicine, 2007.
15. Maddern BR, Reed HT, Ohene-Frempong K, Beckerman RC. Obstructive sleep apnea syndrome in sickle cell disease. Ann Otol Rhinol Laryngol 1989;98:174–8.
16. Islam M, Albustami O, Judy J, et al. Opioid induced sleep disordered breathing in sickle cell patient. J Sleep Disor: Treat Care 2012;1:1.
17. Wang D, Teichtahl H, Drummer O, et al. Central sleep apnea in stable methadone maintenance treatment patients. Chest 2005;228:1348–56.
18. Walker JM, Farney RJ, Rhondeau SM, Boyle KM, Cloward TV, Shilling KC. Chronic opioid use is a risk factor for the development of central sleep apnea and ataxic breathing. J Clin Sleep Med 2007;3:455–61.
19. Javaheri S. Central sleep apnea. Clinics Chest Med 2010;31:235–48.
20. Subramanian S, Guntupalli B, Murugan T, et al. Gender and ethnic differences in prevalence of self-reported insomnia among patients with obstructive sleep apnea. Sleep Breath 2011;15:711–5.
21. Levenson JL, McClish DK, Dahman BA, et al. Depression and anxiety in adults with sickle cell disease: the PiSCES project. Psychosom Med 2008;70:192–6.
22. Hasan SP, Hashmi S, Alhassen M, Lawson W, Castro O. Depression in sickle cell disease. J Natl Med Assoc 2003;95:533–7.
23. Mehta H, Efird JT, Kadali RA, et al. Daytime pulse oximetry measurements may not predict nocturnal desaturations in adult sickle cell patients. Ann Hematol 2013;92:1291–2.
24. Magalang UJ, Dmochowski J, Veeramachaneni S, et al. Prediction of the apnea-hypopnea index from overnight pulse oximetry. Chest 2003;124:1694–701.

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DISCLOSURE STATEMENT

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