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Sleep pattern in a group of patients undergoing hemodialysis compared to control

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Abstract

Background: Patients with chronic kidney disease progress regularly every year to end-stage renal disease and have to undergo dialysis. Sleep disturbances have been reported to be frequent among patients receiving dialysis and contributing to the increase of their mortality and morbidity. The present research aimed to study the sleep pattern in hemodialysis patients and the risk factors associated. This cross-sectional case-control study included 40 subjects divided into 2 groups: 20 cases recruited from Ain Shams University Hospital's dialysis unit and 20 in the control group with normal Pittsburgh Sleep Quality Index score matched for age and sex. Both groups were subjected to overnight polysomnography, and the cases group was assessed by the Pittsburgh Sleep Quality Index to determine their sleep quality.

Results: Nearly all polysomnographic parameters were significantly abnormal in the cases group except for sleep onset latency ($P > 0.05$), showing obstructive sleep apnea and periodic limb movement (P value 0.001). Based on their Pittsburgh Sleep Quality Index score, 30% were classified as good sleepers and 70% as bad sleepers. On comparing both groups, a significant difference was found. Poor sleepers had more worse sleep efficiency (62.9%), spent longer time during their sleep in stage 1 (26.6%) with shorter REM onset latency (113.5 ± 99.5), and had a longer duration of illness with lower serum creatinine level compared to good sleepers.

Conclusions: The prevalence of obstructive sleep apnea and periodic limb movement in hemodialysis patients is high; patients with longer time on dialysis are at more risk of sleep disorders, whereas hemoglobin levels, BUN, and other demographic factors do not seem to play a role in sleep disorder. Hence, patients on hemodialysis need to be screened for sleep disorders so as to improve their mortality and morbidity.

Keywords: Sleep, Hemodialysis, Pattern, Obstructive sleep apnea

Background

End-stage renal disease (ESRD) corresponds to a glomerular filtration rate (eGFR) of < 15 mL/min/1.73 m [1]. It showed variable-elevated incidence across the world where in the USA, the annual incidence rate is 355 per million [2]. In Europe, it reaches 135,000 per year [3]. Saudi Arabia reports a prevalence rate of 5.7% [4]. According to the 9th Annual Report of The Egyptian

Renal Registry, the prevalence in Egypt raised to 483 patients per million [5].

At this stage, survival and quality of life are sustained by kidney replacement therapy, which includes hemodialysis (HD), peritoneal dialysis, and kidney transplantation [6]. Yet, most patients are treated with dialysis due to the scarcity of donor organs and contraindications to transplantation [7, 8].

A global survey was done on nephrologists in over 90 countries to assess the reimbursement for dialysis, suggesting the number of patients receiving HD worldwide was approximately 2,600,000 patients [6] and is expected to be doubled to 5.4 million by 2030 [9]. Hence, HD

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became the main and most widely used replacement treatment for ESRD patients.

With advances in dialysis techniques and medical care, mortality and morbidity rates of patients on regular hemodialysis have markedly declined, yet this is not the only goal for those patients further improvement of their quality of life has become the aim of medical practitioners. And in order to achieve that, both physical and mental health needs to be satisfied and maintained. However, many patients on dialysis suffer from sleep disorders which undoubtedly affect both their physical and mental health status [10] and consequently worsen their quality of life, so this problem needs to be addressed.

The progression to ESRD appeared to be correlated with the development of sleep disorders [11]. This can be explained by the ineffective glomerular filtration occurring at this stage leading to an inability to maintain normal homeostasis affecting various metabolic products including vital bioelements and proteins. This dysregulation in homeostasis might impact sleep in various ways [12].

HD patients report sleep problems more than twice as frequently as healthy control subjects [13]. Sleep disturbances, including insomnia, obstructive sleep apnea (OSA), restless legs syndrome, periodic limb movements disorder, and excessive daytime sleepiness, have been frequently reported in those patients, and they are associated with a negative effect on the quality of life and functional status [14].

A large number of data suggest a bidirectional relationship between OSA and CKD. That is, CKD likely confers an increased risk of OSA, which is related to declining kidney function status, being more prevalent in ESRD patients, compared to CKD patients not on dialysis, and OSA may in turn increase the risk of renal injury [15].

The prevalence of obstructive sleep apnea is higher than in the general population, as reported to occur in at least 50 to 60% of chronic kidney disease patients with ESRD [16, 17]. It affects them tremendously in various ways as the reduction in the airflow occurring during OSA episode leads to acute derangements in gas exchange and recurrent arousals from sleep. This leads to excessive daytime sleepiness, cognitive impairment, decreased work performance, and fall in health-related quality of life. Also as evidenced by many studies that OSA may contribute to the development of systemic hypertension [18], cardiovascular disease [19], and abnormalities in glucose metabolism [20], as the repeated oxygen saturation drops occurring frequently during sleep, increases oxidative stress, and stimulates the sympathetic nervous system [21].

As a result, OSA may aggravate the medical condition of ESRD patients increasing their mortality rate as

proved earlier that cardiovascular disease is the leading cause of death in dialysis patients, and occurrence of sudden death [22] and the presence of diabetes mellitus and poor glycemic control are often associated with an increase of mortality [23, 24]. So, it is not only affecting their quality of life but also poses a threat to their survival, yet it is undiagnosed in many cases.

Hence, the identification and treatment of OSA or other sleep disorders are of clinical significance, as early intervention can diminish daytime fatigue, enhance physical activity, and thus result in improved metabolic control including glycemic control. Diagnosing OSA is therefore important in the management of HD patients, since it is a treatable condition [21].

The purpose of the current study was to explore whether the patients undergoing hemodialysis suffer from sleep disorders, aiming to raise awareness across medical disciplines especially nephrologists and providing them with sufficient knowledge to identify those affected with sleep disorders and implement the treatment in place to enhance their quality of life and improve mortality among those groups of patients by preventing the metabolic derangements caused by OSA.

Methods

This cross-sectional case-control comparative study was conducted in the dialysis units in the nephrology department and Okasha Institute of Psychiatry in Ain Shams University Hospital over 6 months duration during which a convenient sample of 20 patients was chosen fulfilling the inclusion criteria. The procedure of the study was accepted by the ethical committee of Ain Shams University, Cairo, Egypt.

The study included 40 subjects divided into 2 groups:

Cases group: 20 patients receiving regular hemodialysis 3 sessions/week in the dialysis unit fulfilling the inclusion criteria, diagnosed as end-stage renal failure and on regular hemodialysis for at least 6 months, both sexes, age group 18–50 years and giving written informed consent after the purpose of the study was explained, and were given an opportunity to decline to participate in the study without interfering with their medical care. Patients with a history of psychiatric illness before starting dialysis; receiving sedatives, antipsychotics, and antihistaminic; and with severe heart failure, morbid obesity, or respiratory distress were excluded.

Control group: 20 individuals healthy with no medical or psychiatric illness history taking no medication affecting sleep and with global Pittsburgh Sleep Quality Index score ≤ 5 matched with the patient group for age and sex were selected from the employees of Okasha Institute of Psychiatry.

Procedure

All patients included in the study were subjected to complete the clinical examination with the calculation of body mass index (BMI), serum creatinine and BUN, complete blood count, Na and K together with a history of taking about the duration of dialysis, comorbidities, and drug history and assessed for their sleep quality using the Pittsburgh Sleep Quality Index.

Both groups were subjected to the assessment of their sleep pattern using overnight polysomnography (done on the second day of hemodialysis for cases group) in the sleep lab of Okasha Institute of Psychiatry.

Tools used

Pittsburgh Sleep Quality Index (PSQI)

This is a self-rated questionnaire, consisting of 19 questions related to sleep quality in the previous month, including estimates of sleep duration and latency, as well as frequency and severity of specific sleep-related problems. The 19 questions are grouped into seven component scores, each weighted equally on a 0–3 scale. The seven components are summed to yield a global PSQI score (range 0–21); higher scores indicate worse sleep quality [25]. Patients with a global PSQI score > 5 are conventionally defined as “poor sleepers,” whereas those with a score ≤ 5 are considered “good sleepers.” The Arabic version was used which was translated by [26].

Polysomnogram

The apparatus used is Neurofax EEG2110, Digital Electroencephalograph, Nihon Kohden Corporation, Tokyo, Japan. It can provide immediate staging, scoring, and reporting of the PSG night. Hypnographic all night 8 h sleep duration PSG provides the following variables:

- I. Sleep continuity includes sleep latency, sleep efficiency, and arousal index. *Sleep latency*: normally about 15 min. *Sleep efficiency* is usually greater than 90% in the young and decreases somewhat with age [27]. *Arousal index* is the total number of arousals per hour of sleep.
- II. Analysis of sleep architecture includes percentages of NREM-sleep stages (I, II, III, IV, and SWS) to total sleep time. For the age group 41–50 years, which includes our sample age category the normal % varies to be stage I 14%, stage II 50%, and SWS 7% [28].

Periodic Limb Movement Index (PLMI)

It is the number of muscle contractions occurring per hour of sleep. PLMI of more than 5 and less than 25 is considered mild, PLMI of > 25 and < 50 is considered moderate, and > 50 is considered severe [29].

- III. Apneas includes total apnea/hour and desaturation/hour. *Apnea-Hypopnea Index (AHI)*: the total number of complete cessations (apnea) and partial obstructions (hypopnea) of breathing occurring per hour of sleep. AHI can be used to classify the severity of disease as mild 5–15, moderate 15–30, and severe greater than 30 [30].

Respiratory Disturbance Index (RDI)

This is the average number of episodes of apnea, hypopnea, and respiratory event-related arousal per hour of sleep. The American Academy of Sleep Medicine uses the *Respiratory Disturbance Index* to determine the severity of obstructive sleep apnea according to the following range: 5–14.9 for mild, 15–29.9 for moderate, and 30+ for severe [31].

- IV. REM sleep analysis includes *REM percentage* (14% for ages 41–50 years) [28] and *REM sleep latency*.

Statistical methods

Data were analyzed using PASW version 18 (IBM® Corp., Armonk, NY, USA). The normality of data was tested using the D’Agostino-Pearson test, and normally distributed numerical variables were presented as mean ± SD. Numerical data were compared using the unpaired Student *t* test, and qualitative data were compared using the chi-square *t* test or the Fisher exact test. *P* value was considered significant if < 0.05 (S). *P* value was considered non-significant if > 0.1 (NS).

Results

Demographic and clinical data of cases

The mean age of the patients was 41.59 ± 7.12 years; 13 (65%) were male and most of them were married 16 (80%). Their BMI was 25.82 ± 3.05 which classify them within normal to overweight. The duration of illness was 5.55 ± 3.5 years. Ten (50%) had hypertension while 9 (45%) had diabetes mellitus. There was no significant statistical difference between cases and control as shown in Table 1.

Table 1 Socio-demographic characteristics of cases and controls

	Cases	Control	P value
Age (years)	41.59 ± 7.12	39.50 ± 5.41	0.847
Gender	F 7 (35%)	7 (35%)	0.629
	M 13 (65%)	13 (65%)	
Marital state	Single 4 (20.0%)	8 (40%)	0.150
	Married 16 (80%)	12 (60%)	
BMI (kg/m ²)	25.82 ± 3.05	24.32 ± 2.18	0.083
Duration of illness (years)	5.55 ± 3.5		
HTN	10 (50%)	0 (.0%)	< 0.001
DM	9 (45%)	0 (0%)	0.001

BMI body mass index, HTN hypertension, DM diabetes mellitus

Table 2 Comparison between cases and control as regards the sleep study findings (polysomnographic variables)

	Cases	Control	P value
Sleep onset latency	13.3 ± 8.42	15.6 ± 2.29	0.213
Sleep efficiency (%)	68.13 ± 12.85	91.35 ± 1.35	0.000
Sleep stage I (%)	20.85 ± 25.41	3.15 ± .68	0.003
Sleep stage II (%)	36.76 ± 14.56	50.33 ± .43	0.000
SWS (%)	37.60 ± 29.75	22.63 ± .93	0.030
REM sleep (%)	4.80 ± 6.02	26.47 ± 11.40	0.000
REM latency (min)	146.08 ± 102.62	70.60 ± 5.75	0.002
Arousal index (h)	32.08 ± 22.73	.89 ± 1.03	0.000
Sleep total apnea (h)	9.95 ± 11.61	.06 ± .12	0.000
Sleep obstructive apnea (h)	7.23 ± 9.59	.05 ± .11	0.002
Sleep central apnea (h)	1.02 ± 1.38	0	
Mixed apnea (h)	1.66 ± 2.24	.01 ± .02	0.002
AHI/h	13.31 ± 16.98	.02 ± .05	0.001
Desaturations (h)	23.95 ± 22.85	.03 ± .07	0.000
RDI	23.26 ± 23.79	.07 ± .15	0.000
PLM Index	25.37 ± 31.59	.25 ± .31	0.001

Values are expressed as number (%) or as mean ± SD, as appropriate

AHI apnea-hypopnea index, RDI respiratory disturbance index, PLM Index Periodic Limb Movements Index, REM rapid eye movement sleep, SWS slow-wave sleep

Polysomnographic findings in the cases versus control group

On comparing between both groups, a statistically significant difference was found in almost all sleep parameters assessed by the polysomnography except for sleep onset latency (*P* > 0.05) as shown in Table 2.

Where the HD patients suffer from less sleep efficiency (68.13 ± 12.85), they are losing a third of their sleep time. They spend more time in stage I (20%) and SWS stage (37.60 ± 29.75) sleep and significantly less time in stage 2 (36.76 ± 14.56); they are not getting enough REM sleep (4.80 ± 6.02) with increased REM

latency (146.08 ± 102.62 min) and showing frequent arousals (AI; 32.08 ± 22.73/h).

Meanwhile, 65% of cases experience obstructive sleep apnea (AHI; 13.31 ± 16.98/h) with RDI 23.26 ± 23.79 which classify them as having moderate obstructive apnea.

Also, 70% of patients experience frequent periodic limb movements during the night with PLMI 25.37 ± 31.59 categorizing them as periodic limb movements disorder (PLMD) according to the AASM established criterion for PLMD with PLMI cutoff of ≥ 15 [32] with moderate degree (PLMI > 25 < 50). Very few central and/or mixed apneas were recorded among the participants.

Quality of sleep among cases

The patients were classified based on their PSQI score into 2 groups: good sleepers (score less than or equal to 5) and poor sleepers (score more than 5). The percentage of good sleepers among our 20 patients was 30%, and bad sleepers were 70%. Then, both groups were compared according to the clinical, demographic, and polysomnographic variables.

Table 3 showed among different variables, only the duration of illness and serum creatinine level showed a significant difference between both groups with *P* values 0.032 and 0.008, respectively, where poor sleepers had a longer duration of illness and lower serum creatinine levels.

Regarding polysomnographic variables, there were significant differences between good and poor

Table 3 Comparison between the good and bad sleepers groups as regards demographic and clinical variables

	Good sleeper	Poor sleeper	P value
Age	39.2 ± 9.2	43.1 ± 6.0	0.362
Gender	Female 2 (33.3%)	5 (35.7%)	0.664
	Male 4 (66.7%)	9 (64.3%)	
Marital state	Single 2 (33.3%)	2 (14.3%)	0.343
	Married 4 (66.7%)	12 (85.7%)	
BMI	25.1 ± 1.8	26.1 ± 3.5	0.413
Duration of illness	3.5 ± 1.9	6.4 ± 3.7	0.032
HTN	1 (16.7%)	9 (64.3%)	0.070
DM	3 (50%)	6(42.9%)	0.574
Creatinine (mg/dl)	12.1 ± 2.1	8.6 ± 1.9	0.008
Bun (mg/dl)	57.3 ± 7.1	55.4 ± 20.6	0.755
Na (mEq/l)	133.5 ± 5.8	135.6 ± 3	0.438
K (mEq/l)	4.5 ± .3	4.7 ± .9	0.517
Hgb (g/dl)	10.9 ± 1.2	10.4 ± 1.3	0.376
WBCs (10 ³ /μl)	7.2 ± 2.5	7.1 ± 2	0.930
Platelets (× 10 ³ /μl)	166.8 ± 41.6	195.4 ± 64.6	0.256

BMI body mass index, HTN hypertension, DM diabetes mellitus, Hgb hemoglobin

Table 4 Comparison between the good and bad sleepers groups as regards polysomnographic variables

	Good sleeper	Poor sleeper	P value
Sleep onset latency	8.5 ± 8.8	15.1 ± 7.7	0.149
Sleep efficiency (%)	80.4 ± 6.8	62.9 ± 11.2	0.001
Sleep stage I (%)	7.5 ± 7.5	26.6 ± 28.4	0.033
Sleep stage II (%)	33.9 ± 17.6	38.0 ± 13.6	0.623
SWS (%)	51.7 ± 24.0	31.6 ± 30.7	0.141
REM sleep (%)	6.9 ± 7.4	3.9 ± 5.4	0.399
REM latency (min)	222.0 ± 66.2	113.5 ± 99.5	0.012
Arousal index (h)	36.7 ± 15.6	30.1 ± 25.5	0.492
Sleep total apnea (h)	9.8 ± 8.3	10.0 ± 13.1	0.962
Sleep obstructive apnea (h)	5.0 ± 5.0	8.2 ± 11.0	0.375
Sleep central apnea (h)	1.7 ± 1.5	.8 ± 1.3	0.240
Mixed apnea (h)	3.2 ± 2.8	1.0 ± 1.7	0.122
SL hypopnea/h	22.4 ± 20.1	9.4 ± 14.5	0.196
Desaturations (h)	30.0 ± 24	21.4 ± 22.7	0.473
RDI	32.2 ± 26.1	19.4 ± 22.6	0.328
PLM Index	30.7 ± 23.2	23.1 ± 35.1	0.576

Values are expressed as number (%) or as mean ± SD, as appropriate

AHI apnea-hypopnea index, RDI respiratory disturbance index, PLM Index Periodic Limb Movements Index, REM rapid eye movement sleep, SWS slow-wave sleep

sleepers as regards sleep efficiency with *P* value 0.001, stage I percentage *P* value 0.033, and REM onset latency *P* value 0.012, as poor sleepers have worse sleep efficiency (62.9%), spending longer time during their sleep in stage 1 (26.6%) which is light sleep with a shorter REM onset latency (113.5 ± 99.5) min compared to good sleepers, as shown in Table 4.

Discussion

Sleep-disordered breathing is the most common cause of poor sleep in kidney disease patients with a high prevalence in patients on hemodialysis because of compromised upper airway stability [33, 34]. The morbidity and mortality together with the impairment of the quality of life as a consequence of SDB have intensified the need for making early identification and diagnosis.

In the current study, we undertook PSG in HD patients and controls. We found that obstructive sleep apneas were predominant in HD patients. This finding was in line with [13, 35] studies and that of Nicholl and his colleagues' study, whom their data revealed significant increases in the occurrence of obstructive sleep apnea with a prevalence of 57% in the ESRD group [36]. They also concluded that the high incidence of nocturnal hypoxia in chronic kidney disease with ESRD patients could contribute to both the loss of kidney function and increased cardiovascular risk worsening the patient's condition which confirm the importance of detection.

Owing to this high predominance of OSA, HD patients should be considered from the high-risk groups for developing OSA, and therefore, it is crucial to properly point diagnostic attention to them in the medical settings and screen for their existence.

The reasons for the occurrence of sleep apnea are explained by older age and the presence of other risk factors such as hypertension and diabetes [14]. Also, OSA is estimated to be present more in male (20%) than in female (10%) patients with ESRD [37], which is applied to our sample as most of the cases were males (65%) belonging to the older age group (41.59 ± 7.12) having either hypertension (50%) or diabetes (45%).

PSG revealed the occurrence of periodic limb movements among the cases group with moderate degree corroborating with previous studies stating that it is higher in hemodialyzed patients compared to the general population, and it is often in the moderate-severe range [38, 39] and the estimated prevalence is 40–70% among patients on maintenance dialysis [39–41] compared to 7–8% in the general population.

Possible etiologies in the development of PLM in ESRD including our patient group appeared to be related to iron deficiency (Hgb 10.9 ± 1.2 g/dl), the presence of uremia [41], and obstructive sleep apnea conditions [42].

Other PSG findings can be explained by the presence of sleep obstructive apnea and periodic limb movement; the high arousal index (32.08 ± 22.73 vs 16.8 ± 6.2 for this age group), the long REM sleep latency (146.08 ± 102.62 min vs 90 min) [43], and the high percentage of the stage S1 sleep (20% vs 14%) are as a result of frequent arousals caused by sleep apnea and periodic movement of sleep [43], similarly, the low percentage of stage S2 (36% vs 50%) may be explained by sleep fragmentation, obstructive sleep apnea-related arousals, or the increased SWS percentage (37% vs 7%). Finally, the reduced REM frequency (4% vs 14%) could be explained by BaHamam and his colleagues reporting that REM sleep is reduced in patients with severe OSA [44]. These findings were in concordance with Roumelioti et al. showing similar PSG findings in their HD patients when compared to the CKD and control group [45].

Hence, HD patients experience low sleep efficiency, increasing light sleep, and frequent arousals which all contribute to poor quality of sleep and consequently may impact all aspects of their lives.

Using the PSQI questionnaire, cases were divided into good sleepers (score less than or equal to 5) which represented 30% of our studied population and poor sleepers (score more than 5) which represented 70%; the comparison between both groups showed difference as regards the duration of illness being longer in poor sleepers, and serum creatinine was found to

be higher in good sleepers. Serum creatinine level reflects muscle mass and nutritional status; high levels indicate good nutritional status which improves sleep quality as mentioned by Ongan and Yuksel's study finding similar results regarding serum creatinine level where good sleepers had significantly higher creatinine levels than poor sleepers and the risk of poor sleep quality was lower with the increase in serum creatinine levels [46]. Also, Sabbatini et al. reported a significantly higher risk of insomnia in patients with > 12 months on dialysis and higher levels of serum creatinine in the control group vs the insomnia group; they attributed the increased creatinine to the greater number of males and to high intake of protein [47], unlike Iliescu et al. who found no statistically significant relationship between the global PSQI score and serum creatinine level [48]. In the present study, no obvious relationship between anemia and sleep disorders which was reported also by Sabbatini et al.'s study [47] unlikely other studies proved a strong correlation between both [49]. The present study also showed no relationship between other biochemical tests and sleep disorders.

Polysomnography showed a significant difference between good and poor sleepers as regards sleep efficiency (80.4% in good sleepers vs 62.9% in poor sleepers); stage 1 of sleep was longer in poor sleepers with a shorter REM onset latency. Hence, the questionnaire can detect the presence of sleep problems as the subjects in our sample classified as poor sleepers are the cases who experience OSA or PLMD, but the questionnaire cannot diagnose OSA or PLMD. Although the several prediction models and questionnaires have been proposed for diagnosing OSA, the sensitivity of these questionnaires ranges from 80 to 90%, and specificity can be as low as 34%, limiting the utility of this approach [50]. So, PSG remains the criterion standard for the diagnosis of OSA [51].

In hemodialysis patients, PLMS is associated independently with increased estimated cardiovascular and cerebrovascular risk [52] and has been shown to increase the mortality risk [53]. Additionally, cardiac activation is significantly greater when leg movements are associated with obstructive sleep apnea episodes, compared to respiratory events not associated with leg movements [54], and both PLMS and OSA have been associated with daytime sleepiness, impaired cognitive function, and poor vocational abilities. Thus, identification and correction may have the potential to improve the quality of life and clinical outcomes of ESRD patients by reducing those complications associated with sleep disorders.

Conclusions

Obstructive sleep apnea in addition to PLM is common in ESRD patients proved by polysomnography, affecting their sleep quality and increasing daytime sleepiness. These findings should be disseminated among health care professionals in most medical specialties to raise their awareness and provide them with training to help with case identification and initiation of early intervention to foster the well-being of these patients.

Limitation

The study included a small number of patients hence cannot be generalized. Cases groups have HTN and DM which can be confounding factors that may affect sleep quality. Also, in the current study, we did not use any screening questionnaire to exclude any comorbid psychiatric symptoms.

Abbreviations

ESRD: End-stage renal disease; GFR: Glomerular filtration rate; HD: Hemodialysis; OSA: Obstructive sleep apnea; BMI: Body mass index; PSQI: Pittsburgh Sleep Quality Index; PLMI: Periodic Limb Movement Index; REM: Rapid eye movement.

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Authors' contributions

TA designed the work and reviewed the whole study findings. IS analyzed and interpreted the patient data regarding the dialysis. AM collected the data of polysomnography done to the patients and analyzed them. RH analyzed and interpreted the patient data regarding the sleep study and scale and dialysis and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The procedure of the study and the design were accepted and validated by the ethical committee of Ain Shams University, Cairo, Egypt, and informed consent was given by the participants.

Consent for publication

The participants gave consent for using their data in publication.

Competing interests

The authors declare that they have no competing interests.

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