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The quality of life of suspected obstructive sleep apnea patients is related to their subjective sleep quality rather than the apnea-hypopnea index

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Abstract
Purpose The relationship between the severity of the apnea-hypopnea index (AHI) and the quality of life (QOL) in patients with obstructive sleep apnea (OSA) has been inconsistent in previous studies. This study aimed to identify the core factor associated with the QOL of suspected OSA patients and to compare the QOL of subjects with OSA and simple snoring (SS).
Methods Two hundred eighty-five subjects who were clinically suspected to have OSA underwent nocturnal polysomnography (PSG) and completed self-report questionnaires including the World Health Organization Quality of Life Short Form (WHOQOL-BREF) and the Pittsburgh Sleep Quality Index (PSQI). The effects of the clinical and PSG variables on the QOL score were analyzed using multiple stepwise regression analyses, and the QOL of OSA and SS groups was compared.
Results In correlation analyses, the most significant factor that correlated with the QOL of the subjects was the PSQI total score ($p < 0.001$), while the AHI was not related to the WHOQOL-BREF total score. In multiple linear regression analysis, the PSQI total score was the most significant factor associated with the QOL of participants ($p < 0.001$). The mean score of the WHOQOL-BREF did not differ significantly between the OSA group and the SS group.
Conclusion This study suggests that the main factor affecting the QOL of suspected OSA subjects is their subjective sleep quality. We therefore conclude that patients with OSA symptoms estimate their QOL based on their subjective sleep perception rather than AHI.

Keywords Quality of life · Obstructive sleep apnea · Pittsburgh sleep quality index · WHOQOL-BREF

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Introduction

Obstructive sleep apnea (OSA) is characterized by repetitive narrowing or obstruction of the upper airway during sleep that results in apnea or hypopnea. Patients with OSA suffer from poor sleep quality, daytime sleepiness, fatigue, lack of concentration, memory impairment, psychological disturbance, and medical consequences [1, 2].

OSA reportedly impairs the health status and health-related quality of life (QOL). [1, 2] One previous study found that the respiratory disturbance index (RDI) was significantly associated with mobility, cognitive and social functioning, and

health distress in patients with OSA. [3] Some other studies have suggested that OSA patients show a reduction in QOL [4], and that an apnea-hypopnea index (AHI) and arousal index are correlated with a physical functioning subscale for the QOL [5]. However, several previous studies have also found that the severity of QOL impairment is not directly proportional to the severity of OSA [6–9], even though OSA causes the impairment in QOL.

In the clinical situation, many suspected OSA patients consider their subjective sleep quality to be much better than objective PSG data, suggesting that the QOL is not correlated with the AHI. Our previous study—which involved a sample independent from that included in the present study—also suggested that patients with upper airway resistance syndrome (UARS) show more significant psychological symptoms and higher Eysenck Personality Questionnaire scores than do OSA patients [10].

Considering inconsistent findings on the relationship between QOL and AHI of OSA patients in previous studies, we aimed (1) to identify the core factor associated with the QOL in suspected OSA subjects and OSA patients and (2) to compare the QOL of OSA and simple snoring (SS) patients using the WHOQOL-BREF.

Materials and methods

Participant enrollment

In total, 285 subjects aged from 18 to 65 years were recruited in the sleep clinic of Gil Medical Center and Daegu Catholic University Medical Center from March 2011 to February 2016. They were clinically suspected as having OSA, which was indicated by frequent snoring, daytime sleepiness, experience of choking during sleep, and a bed partner witnessing apnea during sleep. All of the subjects met the breathing-related sleep disorder diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (fourth edition, text revision) [11]. The subjects were evaluated by board-certified medical doctors (psychiatrists, ENT doctors, and neurologists) who each had 5 years or more of clinical experience in OSA and sleep medicine.

Subjects with comorbidity of severe medical and surgical conditions and major psychiatric disorders were excluded from this study. Subjects who had previously been diagnosed with OSA, treated with uvulopalatopharyngoplasty, or clinically suspected as having other major sleep disorders such as narcolepsy, restless leg syndrome, rapid eye movement (REM) sleep behavior disorder, or circadian rhythm sleep disorder were also excluded.

We obtained written informed consent from all of the participating subjects, and the study was approved by the institutional review board of both hospitals.

Self-report questionnaire

All of the subjects completed a self-report questionnaire to obtain demographic, medical, and sleep information. The questionnaire asked about their age, sex, occupation, height and weight (to calculate the body mass index (BMI)); history of diseases; consumption of alcohol; smoking; consumption of caffeine; sleeping time; and intake of sleeping medications.

The WHOQOL-BREF was used to evaluate the QOL of subjects. This is an abbreviated version of the WHOQOL-100 QOL assessment [12], asking 26 questions with scores of 1 to 5, where a higher score indicates a better QOL. The WHOQOL-BREF has a total score and scores in the following 4 health domains: physical, psychological, social, and environmental domains. The Korean version of the WHOQOL-BREF was used in our study, which was developed by Min et al. and showed good test–retest reliability, internal consistency, and validity [13]. The subjective sleep quality of the subjects was evaluated with the Pittsburgh Sleep Quality Index (PSQI) [14]. This questionnaire asks 18 questions and presents 7 domains about sleep duration, sleep efficiency, subjective sleep quality, sleep latency, sleep disturbance, use of sleeping medications, and daytime dysfunction during the preceding month with scores from 0 to 3, where a higher score indicates a lower quality of sleep. The Epworth Sleepiness Scale (ESS) was used to assess the daytime sleepiness [15]. The risk of OSA was evaluated using the Berlin Questionnaire and the STOP Questionnaire.

Polysomnography

All subjects underwent in-laboratory, monitored nocturnal PSG. Standard PSG recordings were made in accordance with the American Academy of Sleep Medicine (AASM) recommendations [16]. The PSG used six electroencephalogram leads (F3, F4, C3, C4, O1, and O2), two electrooculogram channels (E1-M2 and E2-M2), three electromyography channels (chin and both anterior tibialis muscles), and one electrocardiography channel. The process of measuring the PSG data, recording the results, and performing the scoring was managed according to the manufacturer's instructions for the COMET and Beehive-7 systems (Grass-Telefactor Corporation). The PSG results were scored based on the criteria in the AASM manual [16], and the presence of hypopnea during sleep was determined as recommended rules in that manual. OSA was defined as an AHI of 5 or more, and SS arbitrarily as an AHI of below 5, and we did not define UARS group. The respiratory effort-related arousal (RERA) was scored by flow limitation using the nasal pressure sensor and plethysmography instead of esophageal pressure for practical reasons. The RDI was defined as the total number of apnea, hypopnea, and RERA episodes per hour. The PSG recordings were analyzed on a computer monitor, and sleep

stages and events were scored visually by experienced PSG technologists based on the criteria of the AASM [16], with all of the PSG data confirmed by sleep specialist medical doctors (K.H.P. and J.E.K.). All of the scorers completed the interscorer reliability program of the AASM (http://www.aasmnet.org/isr/) before starting the study, and their mean score was 92.94 %.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (version 23.0, SPSS Incorporated, Chicago, IL, USA). Descriptive statistics were performed to analyze the demographic data, PSG results, and sleep questionnaire results. The independent Student's t test and chi-square test were used for continuous and dichotomous variables, respectively, when comparing the demographic characteristics, PSG measurements, and QOL scores between the OSA and SS groups. Pearson correlation analysis was used to analyze the correlation among the QOL, clinical-scale scores, and PSG data including respiratory variables, sleep efficiency, and total sleep time. The effects of independent clinical and PSG variables on the QOL score were assessed using multiple stepwise regression analysis after performing correlation analysis. The cutoff for statistical significance was set as $p < 0.05$ (two tailed).

Results

Demographic and clinical (sleep questionnaire) data

Among the 285 subjects who underwent nocturnal PSG, 217 (76 %) subjects were classified into the OSA group and 68 (24 %) into the SS group (Table 1). Age ($p = 0.015$) and the BMI ($p < 0.001$) were higher in the OSA group than in the SS group. The ESS score ($p = 0.014$) and the number of subjects at risk of OSA in the STOP Questionnaire were significantly higher in the OSA group than in the SS group ($p = 0.001$). The PSQI total score did not differ between two groups, while the scores of two domains (sleep efficiency and use of sleeping medication) were higher in the SS group.

PSG data

Table 2 summarizes the results of the PSG measures, comparing between the OSA and SS groups. There were no significant intergroup differences in the total sleep time, sleep latency, sleep efficiency, or waking after sleep onset. The AHI was 1.9 ± 1.4 (mean ± SD) in the SS group and 32.3 ± 22.2 in the OSA group ($p < 0.001$). In terms of sleep architecture, the OSA group showed a higher percentage of sleep at N1 and lower percentages of sleep at N2 and N3. There was no significant intergroup difference in the percentage of REM sleep.

Factors correlated with the QOL score

The clinically important variables that might affect the QOL were calculated in the correlation analysis with WHOQOL-BREF scores (Table 3 and Supplementary Table 1). Among PSG variables, the RDI ($p = 0.024$) and the total arousal index ($p = 0.025$) had significantly positive correlations with the physical domain score of the WHOQOL-BREF. However, the AHI was not correlated with the scores of any domain of the WHOQOL-BREF in all participants (Table 3) and OSA patients (Supplementary Table 1). Age ($p = 0.008$) and the ESS score ($p = 0.022$) had significantly negative correlations with the environmental domain score of the WHOQOL-BREF. Only the PSQI total score showed significant correlations with all of the domain scores as well as the total score of the WHOQOL-BREF in all the participants ($p < 0.001$) and OSA patients ($p \leq 0.001$).

Comparison of the QOL between the SS and OSA groups

The mean scores of the WHOQOL-BREF in the OSA and SS groups are presented in Table 4. The overall QOL score showed a trend toward being higher in the OSA group than in the SS group, but the difference was not statistically significant ($p = 0.085$). The general health, total, and four domain scores of the WHOQOL-BREF did not differ significantly between the two groups.

Major factors that affect the QOL score

The results of multiple linear regression analysis are presented in Table 5. After the correlation analysis, we considered age, sex, PSQI, and RDI as independent variables that could affect the WHOQOL-BREF score. The stepwise method was used for the order of significance in the correlation analysis and variables with multicollinearity were excluded. Only the PSQI was a significant variable ($p < 0.001$) in regression model 1, with an explanatory power of 10.3 % ($R^2 = 0.103$), and the PSQI ($p < 0.001$) and sex ($p = 0.044$) were significant in regression model 2, with an explanatory power of 12.1 % ($R^2 = 0.121$). In the subgroup of OSA subjects, PSQI was the only significant variable ($p < 0.001$) in regression model 1 and the PSQI ($p < 0.001$) and sex ($p = 0.002$) were significant variables in regression model 2.

Discussion

The main finding of this study was that the most important factor influencing the QOL was the subjective sleep quality

Table 1 Demographic and clinical data of all participants, comparing between the SS and OSA groups

Variable	Total (n = 285)	SS (n = 68)	OSA (n = 217)	t^a or χ^{2b} (p value)
Age (years)	44.6 ± 11.3	41.7 ± 11.7	45.5 ± 11.0	$t = -2.5$ ($p = 0.015$)
Sex, female	46 (16.0 %)	16 (23.5 %)	30 (13.8 %)	$\chi^2 = 3.6$ ($p = 0.058$)
ESS total score	9.6 ± 4.7	8.3 ± 4.3	10.0 ± 4.7	$t = -2.5$ ($p = 0.014$)
PSQI total score	8.5 ± 4.4	9.3 ± 5.0	8.3 ± 4.2	$t = 1.5$ ($p = 0.133$)
Subjective sleep quality	1.86 ± 0.8	1.84 ± 0.86	1.86 ± 0.77	$t = -0.23$ ($p = 0.817$)
Sleep latency	1.12 ± 1.0	1.33 ± 1.04	1.06 ± 0.94	$t = 1.93$ ($p = 0.055$)
Sleep duration	1.08 ± 1.0	1.20 ± 1.19	1.04 ± 1.00	$t = 1.01$ ($p = 0.312$)
Habitual sleep efficiency	0.60 ± 1.0	0.84 ± 1.20	0.53 ± 0.89	$t = 2.17$ ($p = 0.031$)
Sleep disturbances	1.57 ± 0.6	1.66 ± 0.66	1.54 ± 0.63	$t = 1.20$ ($p = 0.231$)
Use of sleeping medication	0.25 ± 0.8	0.44 ± 0.99	0.19 ± 0.69	$t = 2.20$ ($p = 0.029$)
Daytime dysfunction	1.55 ± 0.9	1.52 ± 0.89	1.57 ± 0.88	$t = -0.38$ ($p = 0.705$)
High risk of OSA based on the Berlin Questionnaire	255 (89.5 %)	56 (82.4 %)	199 (91.7 %)	$\chi^2 = 2.5$ ($p = 0.113$)
High risk of OSA based on the STOP Questionnaire	254 (89.1 %)	54 (79.4 %)	200 (92.2 %)	$\chi^2 = 11.1$ ($p = 0.001$)
BMI (kg/m^2)	25.8 ± 3.6	24.1 ± 2.7	26.3 ± 3.6	$t = -4.6$ ($p < 0.001$)

Data are mean ± SD or n (%) values

SS simple snoring, *OSA* obstructive sleep apnea, *ESS* Epworth Sleepiness Scale, *PSQI* Pittsburgh Sleep Quality Index, *STOP Questionnaire* snoring, tiredness during the daytime, observed apnea, and high blood pressure, *BMI* body mass index

[a] Independent Student's t test test

[b] Chi-square test

(PSQI score), while the AHI was not a significant factor for the QOL. QOL scores were not correlated with the AHI in the correlation analysis of all subjects and OSA subgroup, and there was no intergroup difference in the QOL score between OSA and SS groups. Our study is in line with several previous studies which found that the AHI was not related to the level of QOL [5–9], sleep perception [17], depression, anxiety symptoms [18, 19], or even sleepiness [7, 8, 18, 20] in the OSA groups.

Our finding could be due to the tendency of OSA patients with higher AHI to feel sleepier at night and fall asleep more easily than do OSA patients with lower AHI or normal people. Such tendency may have impeded their recognition of sleep problem and emotional discomfort [17]. Another possible

Table 2 PSG data of all participants, comparing between the SS and OSA groups

Variable	Total (n = 285)	SS (n = 68)	OSA (n = 217)	t (p value)
Sleep and awake time				
Total sleep time (min)	341 ± 54.2	343 ± 52.2	341 ± 55.0	$t = 0.3$ ($p = 0.751$)
Sleep latency (min)	13.0 ± 19.6	13.8 ± 18.0	12.5 ± 20.0	$t = 0.5$ ($p = 0.620$)
Sleep efficiency (%)	82.7 ± 12.4	82.3 ± 13.6	83.0 ± 12.0	$t = -0.4$ ($p = 0.725$)
WASO (min)	58.1 ± 45.7	62.0 ± 56.0	56.7 ± 42.1	$t = 0.8$ ($p = 0.413$)
Sleep architecture (%)				
Stage N1	28.0 ± 17.2	16.9 ± 8.7	31.4 ± 17.7	$t = -6.5$ ($p < 0.001$)
Stage N2	52.6 ± 15.3	62.1 ± 10.1	50.0 ± 15.4	$t = 6.2$ ($p < 0.001$)
Stage N3	3.3 ± 5.6	4.6 ± 6.1	2.9 ± 5.4	$t = 2.2$ ($p = 0.033$)
Stage R	15.8 ± 6.9	16.4 ± 7.0	15.6 ± 6.9	$t = 0.8$ ($p = 0.424$)
Respiration				
RDI	27.0 ± 22.0	4.0 ± 4.1	34.2 ± 20.3	$t = -10.7$ ($p < 0.001$)
AHI	25.2 ± 23.4	1.9 ± 1.4	32.3 ± 22.2	$t = -11.3$ ($p < 0.001$)
RERA (flow limitation) index	2.2 ± 3.3	2.1 ± 3.9	2.3 ± 3.1	$t = -0.3$ ($p = 0.763$)
Lowest O_2 saturation (%)	79.8 ± 10.6	89.2 ± 3.4	76.8 ± 10.4	$t = 9.7$ ($p < 0.001$)
Movement index				
PLMS (number per hour)	3.3 ± 11.3	4.9 ± 13.7	2.8 ± 10.4	$t = 1.3$ ($p = 0.185$)

Data are mean ± SD values

SS simple snoring, *OSA* obstructive sleep apnea, *WASO* waking after sleep onset, *N1* non-REM stage 1, *N2* non-REM stage 2, *N3* non-REM stage 3, *R* rapid eye movement stage, *RDI* respiratory disturbance index, *AHI* apnea-hypopnea index, *RERA* respiratory effort-related arousal, *PLMS* periodic limb movements during sleep

Table 3 Correlations between PSG data, clinical variables, and WHOQOL-BREF scores

Variable	Domain 1 Physical	Domain 2 Psychological	Domain 3 Social	Domain 4 Environmental	WHOQOL-BREF total score
PSG data					
RDI total	$r = 0.16, p = 0.024$*	$r = 0.08, p = 0.250$	$r = 0.03, p = 0.651$	$r = 0.13, p = 0.062$	$r = 0.12, p = 0.085$
RDI NREM	$r = 0.15, p = 0.031$*	$r = 0.08, p = 0.237$	$r = 0.02, p = 0.756$	$r = 0.11, p = 0.102$	$r = 0.11, p = 0.107$
RDI REM	$r = 0.15, p = 0.026$*	$r = 0.11, p = 0.128$	$r = 0.13, p = 0.065$	$r = 0.18, p = 0.011$*	$r = 0.15, p = 0.027$*
AHI	$r = 0.08, p = 0.180$	$r = 0.02, p = 0.733$	$r = -0.02, p = 0.777$	$r = 0.07, p = 0.232$	$r = 0.05, p = 0.412$
Total arousal index	$r = 0.14, p = 0.025$*	$r = 0.10, p = 0.103$	$r = 0.04, p = 0.482$	$r = 0.07, p = 0.225$	$r = 0.10, p = 0.085$
Demographic and clinical-scale data					
Age	$r = -0.09, p = 0.151$	$r = -0.01, p = 0.816$	$r = -0.08, p = 0.211$	$r = -0.16, p = 0.008$**	$r = -0.08, p = 0.181$
BMI	$r = 0.03, p = 0.574$	$r = 0.08, p = 0.186$	$r = -0.07, p = 0.259$	$r = -0.04, p = 0.543$	$r = -0.02, p = 0.761$
ESS	$r = -0.04, p = 0.526$	$r = -0.10, p = 0.097$	$r = -0.10, p = 0.114$	$r = -0.14, p = 0.022$*	$r = -0.11, p = 0.069$
PSQI	$r = -0.42, p < 0.001$***	$r = -0.34, p < 0.001$***	$r = -0.28, p < 0.001$***	$r = -0.24, p < 0.001$***	$r = -0.36, p < 0.001$***

WHOQOL-BREF World Health Organization quality of life-short form, *RDI* respiratory disturbance index, *NREM* non-rapid eye movement, *REM* rapid eye movement, *AHI* apnea-hypopnea Index, *BMI* body mass index, *ESS* Epworth sleepiness scale, *PSQI* Pittsburgh sleep quality index

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Table 4 WHOQOL-BREF scores of all participants, comparing between the SS and OSA groups

WHOQOL-BREF scale variable	Total ($n = 282$)	SS ($n = 68$)	OSA ($n = 214$)	t (p value)
Overall quality of life	3.3 ± 0.7	3.1 ± 0.8	3.3 ± 0.7	$t = -1.7$ ($p = 0.085$)
General health	2.9 ± 2.6	2.8 ± 1.0	2.9 ± 3.0	$t = -0.4$ ($p = 0.709$)
Total score of the WHOQOL-BREF	74.1 ± 12.0	72.6 ± 11.7	74.5 ± 12.1	$t = -1.1$ ($p = 0.262$)
Domain 1 (physical) total score	21.8 ± 4.3	21.1 ± 4.4	22.0 ± 4.2	$t = -1.4$ ($p = 0.176$)
Domain 2 (psychological) total score	18.5 ± 3.7	17.9 ± 3.7	18.6 ± 3.7	$t = -1.4$ ($p = 0.160$)
Domain 3 (social) total score	9.5 ± 1.8	9.5 ± 1.9	9.5 ± 1.8	$t = -0.3$ ($p = 0.803$)
Domain 4 (environmental) total score	24.2 ± 4.4	23.6 ± 4.3	24.3 ± 4.5	$t = -1.1$ ($p = 0.278$)

Data are mean ± SD values

WHOQOL-BREF World Health Organization quality of life-short form, *SS* simple snoring, *OSA* obstructive sleep apnea

Table 5 Results of multiple stepwise linear regression analysis of the total score of the WHOQOL-BREF

Independent variables	Regression model 1				Regression model 2			
	B	Standard error	β	*p* value	*B*	Standard error	β	*p* value
In all subjects								
Age	–	–	–	–	–	–	–	–
Sex	–	–	–	–	–4.42	2.18	–0.14	0.044
PSQI	–0.91	0.19	–0.32	<0.001	–0.89	0.19	–0.31	<0.001
RDI	–	–	–	–	–	–	–	–
In subjects with OSA								
Age	–	–	–	–	–	–	–	–
Sex	–	–	–	–	–7.79	2.48	–0.21	0.002
PSQI	–1.02	0.19	–0.36	<0.001	–0.97	0.19	–0.34	<0.001
RDI	–	–	–	–	–	–	–	–

Dependent variable: total score of the WHOQOL-BREF, independent variables: age, sex, PSQI, and RDI

WHOQOL-BREF World Health Organization quality of life-short form, *PSQI* Pittsburgh sleep quality Index, *RDI* respiratory disturbance index

explanation is that the combination of comorbid insomnia and OSA can play an important role in emotional suffering among patients with OSA, regardless of the availability of an objective measure such as the AHI [21, 22]. In addition, the paradox that lower oxygen saturation decreases the awareness and perception of sleep problems may also be a contributing factor [23, 24]. Our study indicates that when clinically treating any patients with OSA, the subjective sleep state and distress should be considered as important factors together with the objective severity of the disease, which is AHI. Moreover, OSA patients with low AHI should not be neglected and clinicians should pay attention to patients' subjective sleep perception, potential insomnia, and related distress.

The RDI was positively correlated with the physical health domain score of the WHOQOL-BREF; the physical health QOL score increased as the RDI increased. Since this significance was not robust and disappeared after the regression analysis, we cannot exclude the secondary effect attributable to the relationship between insomnia and QOL or confounding factors.

The SS group showed significantly higher scores in two domains of PSQI and low tendency of QOL score than did the OSA group, although total PSQI score and QOL score did not differ significantly between the two groups. Another possible explanation of this finding could be the effect of the UARS on insomnia and QOL. Previous studies have found that impairments of daily functioning, somatic symptoms, and personality characteristics were greater in UARS patients than in patients with OSA [10, 25, 26]. Because our subjects were arbitrarily divided into two groups based on their AHI, the SS group might have included some UARS patients. Since we did not define the UARS group in our sample, this should be interpreted cautiously.

Our study has a merit in using a global QOL measurement method, the WHOQOL-BREF. The WHOQOL-BREF is a well-validated questionnaire suitable for assessing how patients perceive their health-related QOL or for measuring intervention outcomes in clinical trials [27]. While global measures are suitable for comparison across the groups and interventions [28], disease-specific measures such as Calgary Sleep Apnea Quality of Life Index are good at detecting small changes within subjects. Using a global measurement with general and multidimensional constructs provides comprehensive understanding of the effect of illness [12, 29]. Moreover, global QOL measurements further prevent the overlap of the questions with disease-related scales such as PSQI rather than disease-specific measurements, although WHOQOL-BREF has one sleep question.

The present study was subject to certain limitations. Firstly, because of its cross-sectional design, this study was not able to assess the directionality or the change in the QOL over the disease progression or the course of time. To precisely investigate the effect of subjective sleep measure on the QOL, the longitudinal study design is warranted. Secondly, the characteristics of our subjects—who visited a professional sleep center at a university hospital for OSA-related symptoms—and the lack of a controlled healthy group might affect the generalizability of our results to all suspected OSA patients in the general population. Thirdly, despite using the global QOL measurement, potential systematic errors cannot be completely excluded due to the overlap of the questionnaire.

In summary, this study suggests that the most important factor affecting the QOL of suspected OSA patients is their subjective sleep quality, and that the QOL does not differ between SS and OSA patients. From these findings, we suggest that subjects with OSA symptoms estimate their QOL based on their subjective sleep quality rather than the AHI. Further prospective studies are needed that include larger samples and healthy controls in order to complement the findings of the present study.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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