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Serum levels of NGAL and cystatin C as markers of early kidney dysfunction in patients with obstructive sleep apnea syndrome

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Abstract

Purpose Obstructive sleep apnea syndrome (OSAS) has been recently proposed as an independent risk factor for chronic kidney disease. Cystatin C (Cyst C) and neutrophil gelatinase-associated lipocalin (NGAL) are novel biomarkers for the earlier detection of latent kidney disease. The aim of the study was to assess serum Cyst C and NGAL levels in otherwise healthy OSAS patients and to explore possible associations with sleep parameters.

Methods Consecutive subjects ($n = 96$, 79.2% males), without known comorbidities, with symptoms suggestive of OSAS were included. All of them underwent polysomnography (PSG) and blood examination for the measurement of serum Cyst C and NGAL levels.

Results Based on apnea-hypopnea index (AHI), subjects were classified into two groups: 32 controls and 64 OSAS patients, with no significant differences in terms of age (50.1 ± 11.7 vs 51 ± 12.2 years, $p = 0.747$) and BMI (33.9 ± 8.8 vs 35.9 ± 13.1 kg/m^2, $p = 0.449$). Serum Cyst C and NGAL mean levels were higher in OSAS patients compared to those in controls (1155.2 ± 319.3 vs 966.8 ± 173 ng/ml, $p = 0.001$, and 43.7 ± 23.2 vs 35.6 ± 13.8 ng/ml, $p = 0.035$, respectively). After adjustment for age and BMI in OSAS patients, serum NGAL levels were associated with AHI ($\beta = 0.341$, $p = 0.015$) and minimum oxyhemoglobin saturation during sleep ($\beta = -0.275$, $p = 0.032$), while serum Cyst C levels were associated with percentage of time with oxyhemoglobin saturation < 90% ($\beta = 0.270$, $p = 0.043$), average ($\beta = -0.308$, $p = 0.018$), and minimum ($\beta = -0.410$, $p = 0.001$) oxyhemoglobin saturation during sleep.

Conclusions Higher risk for latent kidney disease in otherwise healthy OSAS patients is indicated. Sleep hypoxia seems to be a significant contributor in the pathogenetic process of renal dysfunction in OSAS.

Keywords Cystatin C · NGAL · Kidney injury · Kidney dysfunction · Obstructive sleep apnea syndrome

Introduction

Obstructive sleep apnea syndrome (OSAS) is a well-known disorder characterized by repetitive episodes of complete or partial upper airway obstruction during sleep, leading to intermittent hypoxia, frequent arousals, and sleep fragmentation [1]. It is a highly prevalent condition with an overall prevalence ranging from 9 to 38% among adults in the general population [2]. Cardiovascular and cerebrovascular diseases are well-established comorbidities among individuals with obstructive sleep apnea and share common pathophysiological mechanisms [3, 4]. Chronic kidney disease (CKD) and its relationship to sleep apnea has also been investigated indicating a possible bi-directional association [5]. In a study, which included 254 patients with CKD [6], OSAS prevalence was 57% among ESRD patients requiring dialysis and 41% among

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patients with eGFR < 60 ml/min not requiring dialysis. Authors concluded that, in this particular population, prevalence of OSAS increased as kidney function declined. Moreover, according to two large epidemiological studies [7, 8] and a cross-sectional study [9], a high prevalence of CKD among OSAS patients has been observed. Proposed mechanisms include fluid overload and pharyngeal narrowing due to rostral fluid shift, as well as altered respiratory drive, due to metabolic acidosis, leading to hyperventilation and hypocapnia that dysregulate respiratory control system and enhance apneic events [5].

Serum cystatin C (Cyst C) is a novel biomarker of kidney function, and a better predictor of early kidney disease compared with serum creatinine [10]. Cyst C is a cysteine proteinase inhibitor produced by nucleated cells all over the human body [11]. It can serve as a useful marker for the evaluation of kidney function because it is freely filtered from the glomerular membrane and then completely reabsorbed without being secreted from the proximal tubular cells [12]. In a recent study [13], increased Cyst C levels were identified in OSAS patients, without known comorbidities, compared to healthy controls. Cyst C levels were associated with indices of hypoxia during sleep, suggesting that OSAS may be implicated in the pathogenesis of latent kidney dysfunction.

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2, is a 25-kDa protein of the lipocalin superfamily, which is partly associated with gelatinase of human neutrophils [14]. NGAL is related to innate immunity through limitation of bacterial infection by iron sequestration [15]. In a mouse model of acute kidney injury, induced by ischemia-reperfusion, levels of NGAL were markedly increased by tubular epithelial cells in response to injury and tubulointerstitial damage, promoting the survival and proliferation of tubular epithelial cells [16]. Accumulative data suggest that NGAL is a reliable marker of CKD progression independently of estimated GFR levels [17]. Plasma NGAL and urine NGAL levels have already been measured in OSAS patients with controversial results [18, 19].

However, available data to date mainly examine the association between CKD and OSA in patients with comorbidities common for both diseases, such as the clusters of metabolic syndromes, namely diabetes, hypertension, obesity, and dyslipidemia. Therefore, the aim of the study was to investigate the association of Cyst C and NGAL in OSAS patients without known comorbidities and to examine the relationship between those markers and polysomnographic and anthropometric parameters.

Materials and methods

Included were consecutive patients referred to the sleep laboratory of our institution with symptoms suggesting sleep-related breathing disorders. The study was carried out in accordance with the Helsinki Declaration of Human Rights. The study protocol was approved by the Institutional ethics committee and all participants provided their informed consent.

Recruitment of patients took place during a 6-month interval between June and December 2016, and the following exclusion criteria were applied: exclusively central sleep apneas on polysomnography (PSG); hypertensive patients; patients with previously diagnosed diabetes mellitus; patients with known kidney, hepatic, or respiratory disease; patients receiving non-steroidal anti-inflammatory drugs (NSAIDs) or any other drugs that could potentially harm renal function; and patients with acute or chronic inflammatory diseases or cancer.

Detailed data regarding previous medical history, current medication use, tobacco smoking, and alcohol consumption were obtained. Clinical examination was performed and anthropometric characteristics were measured. Blood pressure was recorded with subjects in the sitting position using an upper arm mercury sphygmomanometer with arm cuffs of different sizes. Blood pressure was recorded after a 10-min rest, as the average of three consecutive measurements separated by a 1-min interval.

Daytime sleepiness was evaluated using the Greek version of the Epworth Sleepiness Scale (ESS) [20], a self-administered questionnaire evaluating the possibility of falling asleep in a variety of situations [maximum score, 24; score > 10, excessive daytime sleepiness]. Arterial hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or previously diagnosed/under treatment [21]. Participants were categorized as diabetics when fasting plasma glucose levels were ≥ 126 mg/dl [22]. Estimated GFR was calculated using the abbreviated four-variable version of the modification of diet in renal disease (MDRD) formula [23].

As part of the routine clinical evaluation, to exclude pulmonary and cardiovascular disease, pulmonary function testing, arterial blood gas analysis, and a 12-lead electrocardiogram were also performed.

Polysomnography

All patients underwent standard overnight polysomnography (PSG) (Alice® 4, Philips Respironics, Murrysville, PA, USA), attended by an experienced sleep technician, between 22:00 and 06:00 h. A standard montage of electroencephalogram, electro-occulogram, electromyogram, and electrocardiogram signals was used. Thoracic cage and abdominal motion were recorded using inductive plethysmography. Pulse oximetry was registered and airflow was detected using combined oronasal thermistors. The polysomnographic recordings were manually scored according to the 2007 American Association

of Sleep Medicine guidelines for the scoring of sleep and associated events [24]. The proportion of time spent in each stage of sleep was calculated as a percentage of total sleep time. Apnea was defined as a 90% of reduction in airflow for at least 10 s [24]. Hypopnea was defined as a 30% reduction in airflow for at least 10 s in combination with oxyhemoglobin desaturation of at least 3% or an arousal registered by the electroencephalogram [24]. A central apnea was scored in the presence of cessation of airflow for 10 s or longer without an identifiable respiratory effort [24]. The apnea-hypopnea index (AHI) was calculated as the average number of apneas and hypopneas per hour of PSG-recorded sleep time [24]. OSAS was defined as AHI 5/h accompanied by related symptoms and was graded as mild (AHI, 5–14.9/h), moderate (AHI, 15–29.9/h), or severe (AHI ≥ 30/h) [1].

Blood samples and measurements

Venous blood samples were collected the morning after PSG, after at least 8 h of overnight fasting. Blood was immediately centrifuged (10 min at 3000 rpm) and the supernatant was preserved at − 80 °C until analysis. Fasting blood glucose, triglycerides, total cholesterol, high- and low-density lipoprotein, creatinine, urea, and CRP were calculated by a random-access chemistry analyzer (AU640; Olympus; Hamburg, Germany). Cyst C and NGAL serum concentrations were measured by ELISA test using commercially available kits (Biovendor, Czech Republic, for Cyst C and Biovendor-Laboratori Medicina A.S., Czech Republic, for NGAL) according to the manufacturer's specifications.

Statistical analysis

All analyses were performed using version 17.0 of the IBM Statistical Package for Social Sciences (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.). Continuous variables were tested for normality of distribution by the Kolmogorov-Smirnov test. Quantitative data with normal distribution are expressed as mean ± standard deviation (SD) and with skewed distribution as median (25th–75th percentile). The chi-square test was used for comparison of percentages between groups. Correlations were analyzed with Pearson's correlation coefficient, while comparisons between means were explored with the Student *t* test. In case of skewed distribution, the non-parametric Mann-Whitney test was applied. Linear regression analysis with Cyst C and NGAL as the dependent variable was performed. Possible predictors (such as age and BMI) were entered into the regression and then polysomnographic parameters were added to the model. Two-tailed significance was defined at $p < 0.05$ level. Effect sizes (Cohen's *d*, and *f*2) were calculated for all inferential statistics tests, and a post hoc power analysis was conducted using G*Power 3.1. The tests were generally considered satisfactory (0.69 for NGAL and 0.93 for Cyst C).

Results

In total, 96 individuals without known comorbidities (20 females and 76 males), consecutively referred to our sleep unit, were included. Participants were divided according to AHI into two groups: control group (AHI < 5/h), which included 32 subjects (10 females and 22 males); and OSAS group (AHI > 5/h), which included 64 subjects (10 females and 54 males). There were no differences between controls and OSAS patients in terms of age (50.1 ± 11.7 vs 51 ± 12.2 years, $p = 0.747$) and BMI (33.9 ± 8.8 vs 35.9 ± 13.1 kg/m^2, $p = 0.449$). Demographic and anthropometric characteristics of the two groups are presented in Table 1, while sleep characteristics are presented in Table 2.

No significant differences were noted between groups regarding pulmonary function, hepatic function, blood urea, creatinine, eGFR, lipidemic profile, and serum CRP levels. Serum NGAL levels were significantly higher in the OSAS group compared to those in controls (43.7 ± 23.2 vs 35.6 ± 13.8 ng/ml, respectively, $p = 0.035$), as well as serum Cyst C levels (1155.2 ± 319.3 vs 966.8 ± 173 ng/ml, respectively, $p = 0.001$). Results of laboratory analyses are presented in Table 3.

In total, NGAL serum levels were significantly associated with serum Cyst C levels ($r = 0.257$, $p = 0.018$). Moreover, NGAL serum levels were positively associated with AHI ($r = 0.294$, $p = 0.004$) and sleep time at stage 2 (N2) ($r = 0.215$, $p = 0.037$) and negatively associated with minimum oxyhemoglobin saturation during sleep ($r = -0.291$, $p = 0.004$), while Cyst C serum levels were positively associated with ODI ($r = 0.322$, $p = 0.012$), AHI ($r = 0.344$, $p = 0.001$), percentage of time with hemoglobin saturation < 90% ($r = 0.335$, $p = 0.002$), and sleep time at stage 1 (N1) ($r = 0.240$, $p = 0.030$), and negatively associated with sleep stage 3 (N3) ($r = -0.271$, $p = 0.014$), REM ($r = -0.265$, $p = 0.016$), average ($r = -0.373$, $p < 0.001$), and minimum ($r = -0.486$, $p < 0.001$) oxyhemoglobin saturation during sleep.

In the OSAS group, NGAL were positively associated with Cyst C serum levels ($r = 0.259$, $p = 0.039$), while no association was observed between all the examined anthropometric parameters or age and either serum NGAL or Cyst C levels. However, serum NGAL levels were positively associated with AHI ($r = 0.285$, $p = 0.022$) (Fig. 1) and negatively associated with minimum oxyhemoglobin saturation during sleep ($r = -0.252$, $p = 0.044$) (Fig. 2). Additionally, serum Cyst C levels were positively associated with percentage of time with oxyhemoglobin saturation < 90% ($r = 0.256$, $p = 0.041$) (Fig. 3) and negatively associated with average ($r = -0.288$, $p = 0.021$) (Fig. 4) and minimum ($r = -0.394$, $p = 0.001$) oxyhemoglobin saturation during sleep (Fig. 5). No correlation was

Table 1 Comparison of anthropometric characteristics between obstructive sleep apnea syndrome patients and controls

	OSAS patients (AHI > 5/h) $n = 64$	Controls (AHI < 5/h) $n = 32$	p
Gender (male/female)	54/10	22/10	0.076
Age (years)	51 ± 12.2	50.1 ± 11.7	0.747
BMI (kg/m^2)	35.9 ± 13.1	33.9 ± 8.8	0.449
Neck circumference (cm)	43.7 ± 4.3	40.9 ± 3.7	0.01*
Waist circumference (cm)	121.4 ± 14.4	108.8 ± 16.6	0.03*
Hip circumference (cm)	117.5 ± 16.3	116.5 ± 17.5	0.815
WHR	1.04 ± 0.18	0.94 ± 0.09	0.007*
Smoking (%)	50	60.9	0.307

AHI, apnea hypopnea index; *BMI*, body mass index; *OSAS*, obstructive sleep apnea syndrome; *WHR*, waist to hip ratio

*$p < 0.05$

found between serum NGAL and Cyst C levels with daytime sleepiness, expressed as ESS score, and serum CRP levels. Correlations between serum NGAL and Cyst C levels with anthropometric and sleep parameters are shown in Table 4.

After adjustment for age and BMI, serum NGAL levels were significantly associated with AHI ($\beta = 0.341$, $p = 0.015$) and minimum oxyhemoglobin saturation during sleep ($\beta = -0.275$, $p = 0.032$) and serum Cyst C levels were significantly associated

Table 2 Comparison of sleep characteristics between obstructive sleep apnea syndrome patients and controls

	OSAS patients (AHI > 5/h) $n = 64$	Controls (AHI < 5/h) $n = 32$	p
TST (min)	307.5 ± 71.8	299.1 ± 50	0.555
N1 (%)	10.8 (6.7–21.2)	13.2 (6–19.8)	0.621
N2 (%)	65.2 ± 14.9	62.5 ± 12.2	0.380
N3 (%)	2.65 (0–10.1)	15.7 (0.275–20)	0.025*
REM (%)	8.9 ± 6.9	11.3 ± 8.5	0.140
AHI (events/h)	53.9 ± 28.5	2.4 ± 1.3	< 0.001*
Aver SpO_2 (%)	90 ± 3.2	93.9 ± 1.9	< 0.001*
Min SpO_2 (%)	70.5 ± 10.1	88.2 ± 3.6	< 0.001*
$T < 90\%$ (%)	37.8 ± 26.4	5.9 ± 14.7	< 0.001*
ODI (events/h)	56.8 ± 32.2	6.1 ± 3.5	< 0.001*
Arousal index	40 ± 21.3	13.7 ± 6.4	< 0.001*
Sleep efficiency (%)	87.7 (80–94)	86.9 (71.2–91.3)	0.503
ESS score	10.9 ± 5.6	8.8 ± 4	0.071

AHI, apnea-hypopnea index; *Aver SpO_2*, average oxyhemoglobin saturation during sleep; *ESS*, Epworth sleepiness scale; *Min SpO_2*, minimum oxyhemoglobin saturation during sleep; *N1*, sleep stage 1; *N2*, sleep stage 2; *N3*, sleep stage 3; *ODI*, oxygen desaturation index; *OSAS*, obstructive sleep apnea syndrome; *REM*, rapid eye movement; *TST*, total sleep time; *T < 90%*, time with oxyhemoglobin saturation < 90%

*$p < 0.05$

Table 3 Comparison of the laboratory results between obstructive sleep apnea syndrome patients and controls

	OSAS patients (AHI > 5/h) $n = 64$	Controls (AHI < 5/h) $n = 32$	p
FEV_1 (% predicted)	90.9 ± 21.6	98.4 ± 21.5	0.130
FVC (% predicted)	93.6 ± 50.5	97 ± 19	0.728
FEV_1/FVC (%)	82.1 ± 10.3	82.4 ± 8.7	0.894
pO_2 (mmHg)	77 ± 13.6	84.4 ± 12.2	0.013*
pCO_2 (mmHg)	41.1 ± 5.4	40 ± 4.3	0.293
SBP (mmHg)	120 (120–130)	112.5 (110–120)	0.005*
DBP (mmHg)	80 (75–80)	70 (70–80)	0.016*
Glucose (mg/dl)	100.2 ± 16.9	99.3 ± 14.9	0.791
Creatinine (mg/dl)	0.89 ± 0.14	0.90 ± 0.12	0.586
Urea (mg/dl)	35.9 ± 16.2	40.9 ± 18.3	0.177
eGFR (ml/min)	95.3 ± 18.3	89.8 ± 20.2	0.189
Cholesterol (mg/dl)	205.2 ± 44.2	200.6 ± 31.8	0.565
Triglycerides (mg/dl)	181.2 ± 108.7	165 ± 101.2	0.484
LDL-C (mg/dl)	121.6 ± 37.9	114.3 ± 31.5	0.352
HDL-C (mg/dl)	47 ± 12.8	50.5 ± 11.5	0.199
SGOT (U/l)	22.3 ± 6.2	20.3 ± 3.8	0.058
SGPT (U/l)	29.3 ± 14	24.5 ± 11.1	0.094
CRP (mg/dl)	0.55 ± 0.58	0.34 ± 0.36	0.101
Cyst C (ng/ml)	1155.2 ± 319.3	966.8 ± 173	0.001*
NGAL (ng/ml)	43.7 ± 23.2	35.6 ± 13.8	0.035*

CRP, C-reactive protein; *Cyst C*, cystatin C; *DBP*, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FEV1, forced expiratory volume in 1st sec; *FVC*, forced vital capacity; *HDL-C*, high-density lipoprotein-cholesterol; *LDL-C*, low-density lipoprotein-cholesterol; *NGAL*, neutrophil gelatinase-associated lipocalin; *pCO_2*, carbon dioxide partial pressure; *pO_2*, oxygen partial pressure; *SBP*, systolic blood pressure; *SGOT*, serum glutamic oxaloacetic transaminase; *SGPT*, Serum glutamic pyruvic transaminase

*$p < 0.05$

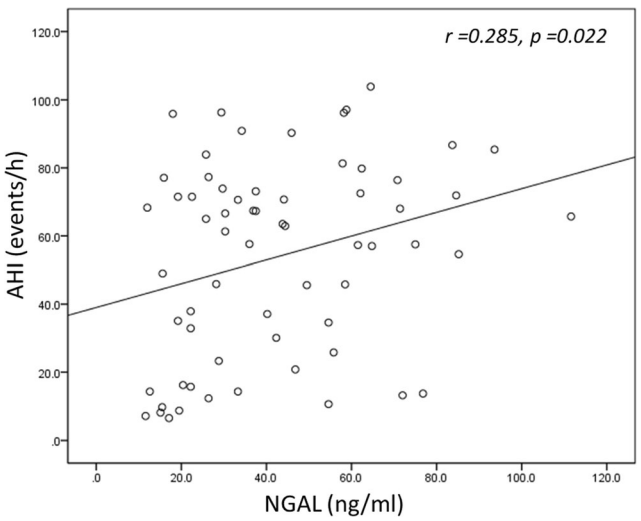

Fig. 1 Correlation between serum neutrophil gelatinase-associated lipocalin (NGAL) levels and apnea-hypopnea index (AHI)

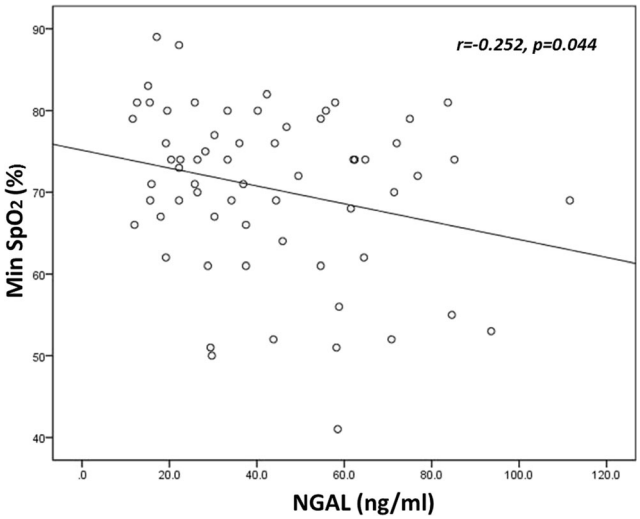

Fig. 2 Correlation between serum neutrophil gelatinase-associated lipocalin (NGAL) levels and minimum oxyhemoglobin saturation during sleep (Min SpO_2)

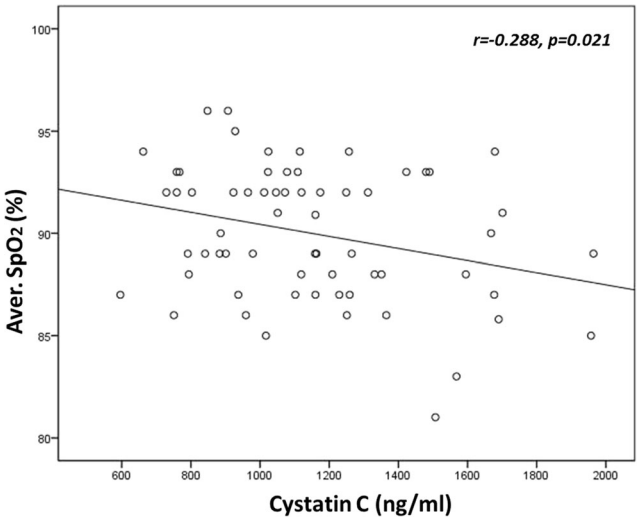

Fig. 4 Correlation between serum Cyst C levels and average oxyhemoglobin saturation during sleep (Aver SpO_2)

with percentage of time with oxyhemoglobin saturation < 90% ($\beta = 0.270$, $p = 0.043$), average ($\beta = -0.308$, $p = 0.018$), and minimum ($\beta = -0.410$, $p = 0.001$) oxyhemoglobin saturation during sleep. After adjustment for age, BMI, neck circumference, waist circumference, and WHR, NGAL serum levels were significantly correlated with AHI ($\beta = 0.521$, $p = 0.016$) and neck circumference ($\beta = -0.508$, $p = 0.041$), while Cyst C serum concentrations were correlated with minimum oxyhemoglobin saturation during sleep ($\beta = -0.334$, $p = 0.030$).

Discussion

Our results suggest an increased risk for latent renal disease in otherwise healthy OSAS patients, compared to age- and BMI-matched healthy controls. Moreover, positive significant association between Cyst C and NGAL serum levels was demonstrated. Both NGAL and Cyst C levels were related with indices of hypoxia during sleep. To the best of our knowledge, this is the first study evaluating at the same time serum levels of NGAL and Cyst C, in OSAS patients without known comorbidities.

In a recent study [18], which included 102 patients assessed with polysomnography, subjects were stratified into four groups according to AHI: controls (AHI < 5/h), mild (5 ≤ AHI < 15/h), moderate (15 ≤ AHI < 30/h), and severe (30/h ≤ AHI) OSAS. Plasma NGAL levels did not differ among groups ($p = 0.16$) while NGAL was positively correlated with AHI ($r = 0.24$, $p = 0.01$), 4% ODI ($r = 0.26$, $p = 0.01$), and length of time spent with $SpO_2 < 90\%$ ($r = 0.23$, $p = 0.02$). Nevertheless, investigators did not exclude patients with hypertension and diabetes mellitus, diseases that contribute to development and progression of renal injury. In the same

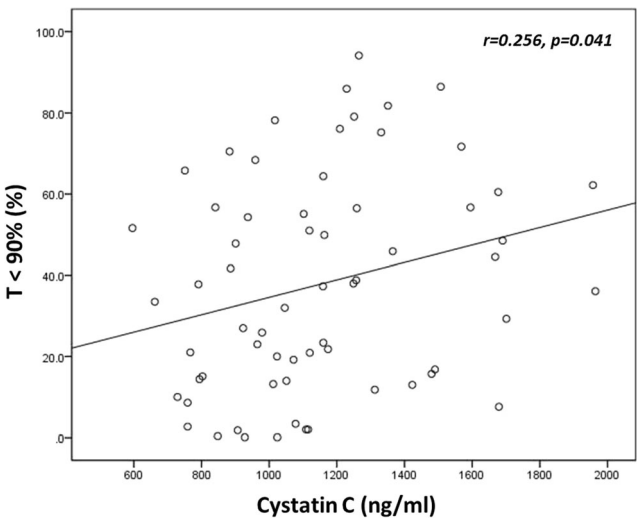

Fig. 3 Correlation between serum Cyst C levels and time with oxyhemoglobin saturation < 90% ($T < 90\%$)

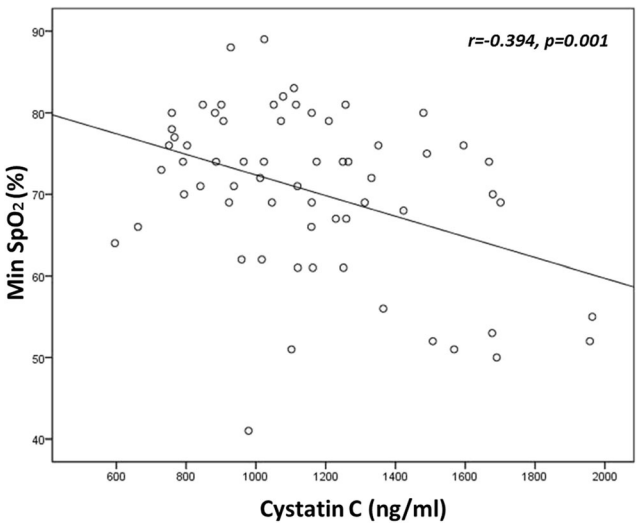

Fig. 5 Correlation between serum Cyst C levels and minimum oxyhemoglobin saturation during sleep (Min SpO_2)

Table 4 Associations between serum NGAL and Cyst C levels and anthropometric, laboratory, and sleep parameters in obstructive sleep apnea syndrome patients

	NGAL		Cystatin C	
	r	*p*	*r*	*p*
eGFR	− 0.150	0.241	− 0.314	0.012*
Creatinine	0.175	0.170	0.341	0.006*
AHI	0.285	0.022*	0.241	0.055
N3	− 0.132	0.307	− 0.254	0.047*
Aver SpO_2	− 0.010	0.938	− 0.288	0.021*
Min SpO_2	− 0.252	0.044*	− 0.394	0.001*
$T < 90\%$	0.014	0.916	0.256	0.041*

AHI, apnea-hypopnea index; *Aver SpO_2*, average oxyhemoglobin saturation during sleep; *eGFR*, estimated glomerular filtration rate; *ESS*, Epworth sleepiness scale; *Min SpO_2*, minimum oxyhemoglobin saturation during sleep; *N1*, sleep stage 1; *N2*, sleep stage 2; *N3*, sleep stage 3; *NGAL*, neutrophil gelatinase-associated lipocalin; *ODI*, oxygen desaturation index; *REM*, rapid eye movement; *TST*, total sleep time; *T < 90%*, time with oxyhemoglobin saturation < 90%; *WHR*, waist to hip ratio

*$p < 0.05$

study, plasma NGAL levels were reevaluated in 25 patients with moderate to severe OSA, after 3 months of CPAP treatment. No significant differences regarding plasma NGAL levels before and after CPAP treatment were observed in the follow-up group, even when good CPAP compliance was considered as a beneficial confounder of lowered NGAL levels (subgroup with good CPAP compliance, 60.5 ± 18.1 vs 64.2 ± 13.9, $p = 0.27$).

In another study, conducted by Maski et al. [19], urinary NGAL/creatinine ratio was evaluated in 49 subjects who were divided into two groups according to OSAS severity: the control group ($n = 16$) and OSAS group ($n = 33$). Subsequently, investigators reevaluated urinary NGAL/creatinine ratio levels in 11 OSAS patients being successfully treated with CPAP for at least 1 month. Moreover, the study population included patients with comorbidities, and specifically those with hypertension and diabetes mellitus. Additionally, BMI values were significantly elevated in the OSAS group compared with those in controls (36.58 ± 11.02 vs 26.81 ± 6.55 kg/m^2 respectively, $p = 0.005$). No significant differences were observed in the urinary NGAL/creatinine ratios between control and OSAS groups (6.34 vs 6.41 ng/mg, respectively, $p = 0.415$) and in the follow-up group of CPAP treatment ($p = 0.776$). There was no correlation between urinary NGAL/creatinine ratio with AHI ($r = -0.106$, $p = 0.467$). Authors concluded that kidney injury may be of glomerular nature and not tubular, as NGAL represents a marker of early tubular kidney injury.

Kiskac et al. [25] recently examined serum NGAL and vaspin levels in 59 subjects, divided into 34 patients with severe OSAS and 25 healthy controls. The study group included individuals without components of the metabolic syndrome. They found no differences in NGAL levels between OSAS and control groups (61.56 ± 18.21 vs 68.53 ± 20.10 ng/ml, respectively, $p = 0.170$).

Cyst C is also a reliable marker of latent kidney injury. Previous studies [26–28] evaluating the role of Cyst C in OSAS concluded that Cyst C is increased among patients with OSAS. However, in most of these studies, enrolled patients had also diabetes and hypertension, leading to the assumption that results could be confounded by means of the metabolic syndrome. Zhang et al. [29] measured serum Cyst C in a younger population (mean age 32.5 ± 5.19 years) of 98 subjects without comorbidities. Authors found increased levels of Cyst C in patients with severe OSAS compared to healthy controls (0.87 ± 0.12 vs 0.74 ± 0.1 mg/l, respectively, $p < 0.05$). Cyst C was associated with AHI ($r = 0.319$, $p = 0.001$) and ODI ($r = 0.279$, $p = 0.005$). Nevertheless, BMI was significantly higher in the OSAS compared to the control group (29.69 ± 3.81 vs 26.42 ± 3.10 kg/m^2, $p < 0.05$). In our study, included OSAS patients did not differ in terms of BMI; thus, obesity was excluded as a possible confounding factor.

In accordance with previous studies, our results suggest that hypoxia and severity of nocturnal oxygen desaturation appear to be key elements in the process leading to the evolvement of renal disease in OSAS. In a recent cross-sectional study, Marrone et al. [9] reported that, in OSAS patients, each unit of decrease in the minimum oxyhemoglobin saturation predicted 2% greater probability of CKD (OR 0.977, 95% CI 0.967 to 0.987). The investigators concluded that severe nocturnal oxygen desaturation, even of limited duration during sleep, may represent an independent predictor of CKD among OSAS patients. Similar to our results, another cohort study conducted by Ahmed et al. [30], which included 858 subjects, demonstrated that nocturnal hypoxia was associated with increased risk for accelerated kidney disease, as estimated by a decline in eGFR ≥4 ml/min/1.73 m^2 per year (OR 2.89, CI 1.25–6.67), indicating that nocturnal hypoxia plays an important role in the development of CKD among OSAS patients. OSAS patients presented in our sleep clinic were all symptomatic and referred with sleep apnea complaints. We did not find whether non-symptomatic OSAS patients could be potentially exposed to latent kidney disease as expressed by increased serum levels of NGAL and Cyst C. However, no significant differences were observed between patients and controls in terms of daytime sleepiness as expressed by ESS.

Of note, in our study, OSAS patients had a reduced proportion of N3 sleep stage compared with non-apneic subjects, a result which is expected in obstructive sleep apnea due to fragmented sleep. Cyst C levels were found to be inversely associated with percentage of N3 stage ($r = -0.254$, $p = 0.047$). Both increased Cyst C and disturbed sleep architecture with sleep deprivation and reduced slow-wave sleep (SWS) reflect, through alterations in metabolism, an increased risk for

development of diabetes. Diabetes represents an established risk factor for CKD [31]. Thus, our results indirectly represent aspects of the comorbid associations between OSAS, kidney function, and metabolism.

Finally, results of our study suggest that OSAS patients could be potentially exposed to latent kidney injury as expressed by increased levels of novel biomarkers, NGAL and Cyst C. Cyst C seems to be of better diagnostic value, because of its stronger correlations with indices of hypoxia during sleep in our study, as well as its broad availability and application in current recommended equations for estimation of glomerular filtration rate (eGFR) and detecting better than serum creatinine loss of kidney function [32]. Early detection of patients with concomitant OSAS and CKD is required in order to identify those CKD patients who might benefit from treatment of OSAS and to prevent further deterioration of kidney disease. It is well established that OSAS is effectively treated with CPAP and should be performed to all patients improving symptoms and quality of life and reducing consequences of OSAS [33, 34]. Interestingly, recent studies reported that CPAP therapy has a beneficial impact on kidney function either in terms of improving renal RAAS activity or in preventing eGFR decline [35–37]. Therefore, clinicians should be aware of this mutual association between OSAS and CKD and identify and treat properly both conditions in order to reduce potential development or progression of CKD.

Our results are subject to certain limitations. A post hoc power analysis has showed that the sample size used in this study was sufficient to detect differences between patients and controls (for both Cyst C and, to a smaller extent, NGAL). However, verification of these results in larger cohorts is necessary to allow extrapolation of our results to all OSAS patients. Another limitation of the study was that we included only individuals who were referred to our sleep laboratory with a suspicion of symptomatic OSAS, and we did not randomly select community-dwelling subjects. However, our aim was to explore whether OSAS patients are at higher risk for kidney disease, in a latent state and before CKD could be established. Moreover, previous studies showed that CKD patients with and without sleep apnea, either coming from the sleep clinic or from the general population, often report similar symptoms suggestive of sleep-disordered breathing, and that available screening tools failed to diagnose OSAS among CKD patients [38–40]. Thus, objective sleep evaluation is required in order to diagnose CKD patients with comorbid OSAS. Additionally, our study was cross-sectional on design and we did not investigate longitudinally whether the effect of CPAP treatment on serum Cyst C and NGAL levels could be beneficial in terms of reduced levels of those markers in order to be performed as monitoring tools of CPAP improvement upon kidney function in future studies. Interestingly, in a previous study including 39 patients with severe OSAS, 3 months of CPAP treatment significantly reduced serum Cyst C levels (0.87 ± 0.18 before treatment vs 0.77 ± 0.21 mg/l after treatment, $p < 0.001$) [28]. Furthermore, our data come from middle-aged adults (51 ± 12.2 years for OSAS patients and 50.1 ± 11.7 years for controls), thus cannot be directly extrapolated to elderly individuals. Another limitation was that males were more represented than females; a fact that reflects the epidemiological distribution of consecutively examined subjects in a sleep laboratory as well as diagnosed OSAS patients. Finally, study participants were not tested for albuminuria. However, increased serum Cyst C levels and albuminuria are considered independent risk factors for CKD and the former may show renal damage in patients without urinary albumin [41].

In conclusion, NGAL and Cyst C levels were found to be increased among OSAS patients without comorbidities in comparison to age- and BMI-matched, otherwise healthy, subjects. OSAS constitutes an independent risk factor of CKD, and hypoxia during sleep appears to be a key element factor in the pathogenesis of renal dysfunction in OSAS. Thus, further research is necessary in order for early diagnosis and treatment of renal disease in OSAS patients to be accomplished.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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.

Informed consent Informed consent was obtained from all individual participants included in the study.

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