RESEARCH

Elevated PAPP-A Levels In Patients With Obstructive Sleep Apnea Syndrome

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Abstract: Aim of this study was to evaluate serum concentration of Pregnancy-Associated Placental Protein-A (PAPP-A) in obstructive sleep apnea syndrome (OSAS) and to examine the relationship of PAPP-A with disease severity and body mass index (BMI). 60 OSAS patients and 21 control subjects included. All study participants were evaluated with polysomnograpic data, BMI, serum lipid, insulin, glucose and PAPP-A levels.PAPP-A values were higher in OSAS group than control group. When the groups were examined according to apnea-hypopnea index (AHI), PAPP-A level of severe OSAS group was determined to be significantly higher than that of control and mild and moderate OSAS groups. As the severity of OSAS increased, the level of PAPP-A increased. In ROC analysis applied to PAPP-A levels of the OSAS and control groups, the optimum cut-off point for serum PAPP-A levels in this study population was 2.38 ng/ml. A positive correlation was determined between PAPP-A levels, BMI and insulin levels. PAPP-A could be a clinical marker for the risk of metabolic syndrome and cardiovascular events in OSAS. Since OSAS patients have a high risk of developing cardiovascular disease, it would be useful to assess prognostic data provided by PAPP-A.

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a common sleep disorder in which the respiratory system is affected, and is characterised by daytime sleepiness, recurrent apnea and hypopnea, and cardiopulmonary modifications. OSAS patients experience repeated attacks of interrupted respiration, thereby exposing the cardiovascular system to cycles of hypoxia, extreme negative intrathoracic pressure and stimulation[1-3]. OSAS has been shown to be a significant risk factor for cardiovascular diseases such as ischaemic heart disease, arrythmia and hypertension, in approximately 2% of females and 4% of males[4-7].

Pregnancy-associated plasma protein-A (PAPP-A) is a placental protein found in abundance in the circulation of pregnant women[8]. It is commonly used in the screening for aneuploidy in the first trimester of pregnancy[9]. PAPP-A is a zinc metalloproteinase in the insulin-like growth factor (IGF) system[10]. PAPP-A has a primary biological function of proteolytic differentiation as an IGF-binding protein (IGFBP) and this leads to local expression of IGFs close to the cell surface[11]. The binding of PAPP-A to proteoglycans on the cell surface results in proteolytic cleavage of IGFBP-4 occurring close to the IGF receptor[12], thereby strengthening the likelihood that released IGF leads to receptor signaling[13]. The IGF system is considered to play a role in the development of cardiovascular disease[14] and the potential relationship between PAPP-A and cardiovascular disease[15-17].

PAPP-A appears to be an important regulator in the IGF system and has an accepted role in the cardiovascular system physiology[18]. In addition a relationship has been reported between the long-term risk of cardiovascular disease and OSAS related to the IGF system. The aim of this study was to search whether or not there was a difference in serum PAPP-A levels between OSAS patients and a control group. Examinations were made of serum PAPP-A levels in relation to BMI and the metabolic profile to determine the relationships between various markers related to cardiovascular disease.

MATERIALS and METHODS

Approval for the study was granted by the Ethics Committee and the Institutional Review Board of Kahramanmaras Sütcü Imam University Medical Faculty (07.11.2018/18). All the study participants provided written informed consent. The study included 60 patients with OSAS and a control group of 21 healthy individuals. All the study subjects were evaluated in the Sleep Disorders Center of the Chest Diseases Department of Kahramanmaras Necip Fazil City Hospital.

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Patients were not included in the study if they have had any of the following disease or disorders: < 18 years, presence of CVD, congenital abnormalities of the head and neck region, neurologic or metabolic diseases (epilepsy, diabetes mellitus), malignancy, sleep disorders other than OSAS (e.g. restless legs syndrome, insomnia) and any pulmonary disorders. According to the PSG results, patients with Apnea Hypopnea Index (AHI) < 5 were included in the control group. OSAS was categorised using the criteria of the American Academy of Sleep Medicine Task Force (AASM)[19]:

- Normal respiration; AHI < 5 events/ hour,
- Mild OSAS: AHI 5 -15 events/ hour,
- Moderate OSAS: AHI 15 - 30 events/ hour,
- Severe OSAS: AHI > 30 events/ hour.

The Apnea-Hypopnea Index (AHI) is calculated by dividing the total sleep hours by the number of apnea-hypopnea events and is stated as events/ hour.

The OSAS patients and the control group subjects were classified according to Body Mass Index (BMI) as:

- Normal: BMI < 25
- Overweight: BMI 25-30
- Obese: BMI ≥ 30.

The subjects in each group had similar demographic characteristics and body mass index (BMI) values. Fasting blood glucose, fasting insulin, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides (TG) measurements were performed on the serum of each subjects. Insulin resistance was measured using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) with the following formula: fasting insülin (mIU/L) × fasting plasma glucose (mg/dL) / 405.

The serum PAPP-A concentration was analysed using commercial kits for the enzyme-linked immunosorbent assay (ELISA) method according to the manufacturer's instructions (Shanghai Coon Koon Biotect Co, Ltd, China). Measurements were taken using an automatic ELISA reader (Thermo Scientific, FINLAND) and a computer program (Scanlt for Multiscan FC 2.5.1). Absorbancy of each well was defined as 450nm. The standard curve was drawn using mean absorbancy of standards (Y) and known concentration of standards (X). The results were reported as PAPP-A concentration (ng/mL) in the samples.

In a study by Cengiz et al.[20] of patients with OSAS, the difference in PAPP-A level between OSAS groups and the control group was detected at 96.7%. Based on that study, the power analysis of this study determined that for test power to be 0.95 with error accepted as 0.05, each group required at least 5 patients.

Polysomnography recording took place in sleep laboratory in a city hospital for one night. A standart procedure was used. Signal data from electroencephalogram (EEG), electromyogram (EMG), electrocardiogram (ECG) and electrooculogram (EOG) PSG channels were recorded. All electrodes were replaced without a common reference[19]. A trained medical doctor with experience scoredall records using standart AASM rules[9,21,22].

All data were analyzed using IBM SPSS Statistics version 20.0 (IBM, Armonk, NY, USA). Kolmogorov Smirnov test was utilized to assess the data conformity to normal distribution and homogeneity was examined with the Levene test. Descriptive statistics were expressed as mean ± standard deviation (SD) or median (minimum-maximum) values categoric data as percentages (%). Differences between the patient and control groups were evaluated with the Mann Whitney U-test. Differences within the groups according to BMI were examined with the Kruskall Wallis test. For the comparison of parameters between the OSAS groups, the Kruskal Wallis test and non-parametric posthoc test were used. The ROC curve was operated for diagnostic tests of PAPP-A. Correlations between the variables were evaluated through the Spearman Correlation test.

RESULTS

The study included a total of 81 individuals, as a control group of 21 subjects and 60 OSAS patients, stratified according to the AHI scores as 18 mild, 18 moderate and 24 severe OSAS. It was determined that the BMI values of OSAS patients were higher than the control group [26.4 (19.6 - 39.9) kg/m2 and 24.7 (17.9 - 33.3) kg/m2, respectively, p= 0.029]. The median PAPP-A values were significantly higher in the OSAS group (5.5 ng/mL; range 2.7-36.2) than in the control group (1.9 ng/mL; range, 1.3 - 5.4) (p= 0.000). When the PAPP-A levels of the OSAS patients were evaluated according to the BMI subgroups of normal, overweight and obese, no significant difference was determined (p= 0.687). In the control group, when evaluation of PAPP-A levels was made according to the BMI subgroups, the PAPP-A levels of the obese group were higher than the normal and overweight subgroups (p= 0.027). The results of the comparative analysis between the OSAS and control groups related to BMI for all the parameters are shown in Table 1 and Table 2.

Table 1. Demographic, clinical and biochemical characteristics patients with OSAS and control groups.

	CONTROL (n= 21)	OSAS (n= 60)	P
Age (years)	44.6±10.78	51.0±10.1	0.014*
BMI (kg/m^2)	24.7 (17.9-33.3)	26.4 (19.6-39.9)	0.029*
AHI (/h)	2.6 (0-4.7)	23.7 (5.8-97.4)	0.000*
FG (mg/dL)	96 (82-108)	98 (72-167)	0.385
Insulin (U/L)	13.8 (3.9-25.0)	18.1 (6.4-42.8)	0.052
HOMA-IR	3.3 (0.8-5.9)	4.3 (1.4-16.3)	0.036*
Cholesterol (mg/dL)	171±42	188±39	0.223
TG (mg/dL)	190 (48-233)	175 (62-421)	0.511
LDL (mg/dL)	140±27	127±24	0.063
HDL (mg/dL)	42 (32-59)	44 (26-70)	0.493
PAPP-A (ng/mL)	1.9 (1.3-5.4)	5.5 (2.7-36.2)	0.000*

BMI: Body Mass Index; AHI: Apnea Hypopnea Index; FG: Fasting blood glucose; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; HOMA-IR: Homeostatic model assessment indicator of insulin resistance; PAPP-A: Pregnancy-associated placental protein-A

*p value is based on Mann Whitney U-test

When the groups were examined according to AHI, the PAPP-A levels of the severe OSAS group were determined to be significantly higher than those of the control group and the mild and moderate OSAS groups (p= 0.000). As the severity of OSAS increased, so the PAPP-A level increased. The laboratory test results and demographic characteristics according to OSAS severity are shown in Table 3.

The ROC curve was drawn for serum PAPP-A (Figure 1). High values indicated the presence of OSAS, with a cut-off point of 2.38, sensitivity of 1.00, and specificity of 0.571 for PAPP-A (ROC AUC= 0.886; 95%CI= 0.804 - 0.967; p= 0.000).

PAPP-A levels were positively correlated with age (p= 0.010, r= 0.285), BMI (p= 0.001, r=0.358), AHI (p= 0.000, r= 0.639) and insulin levels (p= 0.043, r= 0.226) (Figure 2) . When all the patients were evaluated, no significant correlation was determined between the PAPP-A levels and other parameters (p> 0.05).

The PSG results of the control group and the OSAS subgroups are shown in Table 4. A positive correlation was determined between PAPP-A and sleep efficiency (%), oxygen desaturation index (h) and average heart rate (bpm), and a negative correlation was determined with minimum oxygen saturation (%) (Table 5).

Table 2. Parameters of control and OSAS subgroups according to BMI.

	CONTROL (n= 21)			OSAS (n= 60)			p[a]	p[b]
	BMI < 25 (n= 10)	BMI 25 - 30 (n= 8)	BMI ≥ 30 (n= 3)	BMI < 25 (n= 23)	BMI 25 - 30 (n= 18)	BMI ≥ 30 (n= 19)		
Age (years)	43.2 ± 1.1	46.5 ± 11.9	44.7 ± 8.5	54.4 ± 11.1	46.1 ± 10.6	51.6 ± 5.8	0.948	0.042*
AHI (h)	2.6 (0.9 - 3.7)	2.3 (0 - 4.6)	4.5 (0.8 - 4.7)	23.5 (5.8 - 78.1)	17.4 (8 - 82.1)	35.1 (16.3 - 97.4)	0.524	0.019
FG (mg/dL)	99 (82 - 103)	96 (87 - 108)	96 (94 - 100)	98 (72 - 143)	96 (88 - 167)	98 (76 - 146)	0.988	0.697
Insulin (U/L)	11.9 (3.9 - 25.0)	13.5 (10.4 - 20.4)	21.4 (21.3 - 23.9)	18.1 (8.1 - 23.3)	13.9 (6.4 - 25.4)	22.1 (12.7 - 42.8)	0.041*	0.000*
HOMA-IR	2.9 (0.8 - 5.7)	3.2 (2.2 - 4.8)	5.1 (4.4 - 5.9)	4.3 (1.9 - 6.0)	3.1 (1.4 - 6.1)	4.9 (3.1 - 16.3)	0.039*	0.004*
Cholesterol(mg/dL)	160 ± 49	193 ± 20	147 ± 51	195 ± 38	193 ± 42	175 ± 36	0.092	0.046*
TG (mg/dL)	195 (48 - 233)	148 (70 - 213)	197 (136 - 216)	200 (62 - 421)	159 (84 - 279)	158 (73 - 246)	0.667	0.316
LDL (mg/dL)	138 ± 29	142 ± 22	139 ± 41	132 ± 27	127 ± 22	120 ± 21	0.884	0.306
HDL (mg/dL)	41 (32 - 59)	42 (37 - 50)	44 (44 - 47)	42 (31 - 70)	47 (31 - 64)	41 (26 - 51)	0.314	0.092
PAPP-A (ng/mL)	1.6 (1.3 - 5.2)	3.3 (1.6 - 5.4)	4.1 (1.6 - 4.9)	4.7 (2.9 - 17.6)	5.6 (2.7 - 36.2)	5.3 (4.5 - 15.2)	0.027*	0.712

BMI: Body Mass Index; AHI: Apnea Hypopnea Index; FG: Fasting blood glucose; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; HOMA-IR: Homeostatic model assessment indicator of insulin resistance; PAPP-A: Pregnancy-associated placental protein-A
[a] p Difference between BMI subgroups in the control group: Kruskal– Wallis test
[b] p Difference between BMI subgroups in the OSAS group: Kruskal– Wallis test

Table 3. The laboratory and demographic characteristics of the study population based on OSA stage.

	Control group (n= 21)	Mild OSA (n= 18)	Moderate OSA (n= 18)	Severe OSA (n= 24)	P value
Age (years)	44.6±10.7	52.2±9.0	49.4±11.4	51.3±10.0	0.065
BMI (kg/m^2)	24.7 (17.9-33.3)	24.6 (19.7-29.8)	30.0 (19.7-34.3)	31.0 (23.5-39.9)	0.000*
AHI (/h)	2.6 (0-4.7)	8.8 (5.8-14.9)	21.9 (15.7-28.4)	53 (32-97)	0.000*
FG (mg/dL)	96 (82-108)	98 (72-130)	98 (76-143)	98 (83-167)	0.710
Insulin (U/L)	13.8 (3.9-25.0)	14.7 (6.4-23.3)	17.6 (10.324.2)	20.7 (12.6-42.8)	0.000*
HOMA-IR	3.3 (0.8-5.9)	3.9 (1.4-6.0)	3.8 (2.5-7.7)	4.9 (2.4-16.3)	0.001*
Cholesterol (mg/dL)	171±42	201±40	162±25	198±39	0.004*
TG (mg/dL)	190 (48-233)	191 (65-205)	151 (62-207)	198 (91-421)	0.028*
LDL (mg/dL)	140±27	130±26	123±16	128±28	0.218
HDL (mg/dL)	42 (32-59)	44 (31-70)	46 (26-61)	42 (28-62)	0.702
PAPP-A (ng/mL)	1.9 (1.3-5.4)	3.6 (2.7-36.2)	5.8 (3.5-14.6)	5.9 (4.2-17.6)	0.000*

BMI: Body Mass Index; AHI: Apnea Hypopnea Index; FG: Fasting blood glucose; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; HOMA-IR: Homeostatic model assessment indicator of insulin resistance; PAPP-A: Pregnancy-associated placental protein-A
*p value is based on Kruskal-Wallis Test.

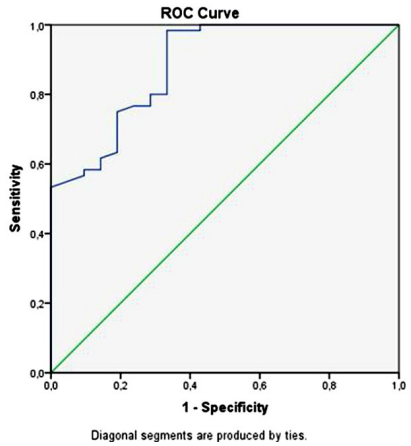

Figure 1. ROC analysesof PAPP-A levels between the OSAS and control groups

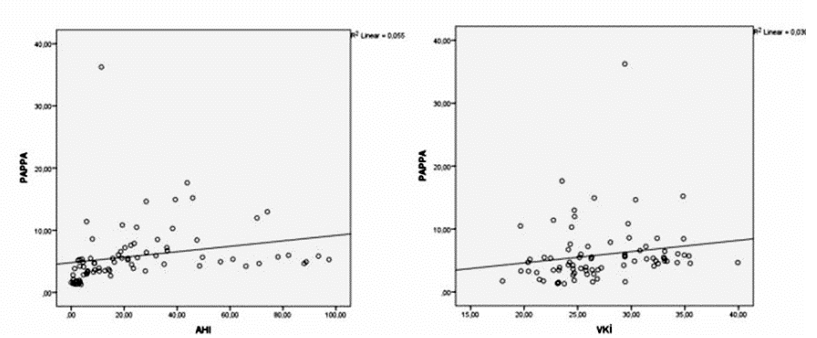

Figure 2. Serum PAPP-A levels were signaficantly positively correlated with apnea hypopnea index (AHI) and Body mass index (BMI)

Table 4. Result of sleep study (polysomnography) measurements of the study population based on OSA stage.

	Control group (n= 21)	Mild OSA (n= 18)	Moderate OSA (n= 18)	Severe OSA (n= 24)	P value
AHI (/h)	2.6 (0-4.7)	8.8 (5.8-14.9)	21.9 (15.7-28.4)	53.0 (32.0-97.40)	0.000*
Oxygen desaturation index (h)	4.0 (0-20.0)	10.3 (2.5-29.3)	28.4 (7.6-54.6)	49.3 ((13.1-116.3)	0.000*
Minimum oxygen saturation (%)	90 (72-95)	86 (74-93)	83 (63-92)	77 (27-88)	0.000*
Sleep efficiency (%)	77.1 (56.7-96.3)	85.8 (52.7-98.1)	85.6 (70.6-96.4)	85.6 (42.0-99.7)	0.060
REM (%)	11.8 (6.7-25.1)	11.3 (3.2-18.6)	12.3 (2.7-23.0)	11.2 (0.3-28)	0.183
Non-REM stage 1 (%)	14.0 (4.7-37.8)	11.8 (3.3-21.5)	13.3 (5.6-22.7)	13.4 (2.4-49.8)	0.491
Non-REM stage 2 (%)	54.2 (35.9-72.6)	54.5 (30.6-84.2)	56.1 (46.2-77.3)	58.9 (33-82.1)	0.094
Non-REM stage 3 (%)	18.6 (1.5-33.4)	20.9 (6.6- 40.0)	16.8 (4.6-26.3)	15.0 (1.9-33.1)	0.082
Average Heart Rate	64 (20-143)	60 (40-88)	63 (48-94)	70 (53-89)	0.000*

*P value is based on Kruskal-Wallis Test.
Group with different statistical significance according to the posthoc test are shown in superscript roman numbers.
AHI, apnea hypopnea index; REM, rapid eye movement

Table 5. The correlations between PAPP-A levels and Polysomnography parameters.

Parameters	R*	p
AHI (/h)	0.639	0.000*
Oxygen desuration index (h)	0.495	0.000*
Min. oxygen saturation (%)	-0.409	0.000*
Sleep efficiency (%)	0.399	0.000*
REM (%)	-0.108	0.338
Non-REM stage 1 (%)	0.043	0.700
Non-REM stage 2 (%)	-0.030	0.789
Non-REM stage 3 (%)	0.097	0.389
Average Heart Rate	0.222	0.046*

*Spearman correlation analysis.

DISCUSSION

The data obtained in this study showed significantly higher PAPP-A levels in the OSAS patients than in the control group, and with increasing severity of OSAS increased, so the PAPP-A level also increased. The BMI values of the OSAS patients were also found to be higher than those of the control group, severe OSAS patients had even higher BMI values, and the PAPP-A levels were seen to increase in correlation with BMI. The PAPP-A levels were also determined to be correlated with insulin levels, suggesting that PAPP-A could be a clinical marker of increased risk for metabolic syndrome and cardiovascular events in OSAS patients. The use of PAPP-A, which provides information related to a high risk for the development of cardiovascular disease, could be useful in the evaluation of cardiovascular risk.

Although PAPP-A was first detected in the serum of pregnant women[8], studies have recently reported high PAPP-A in the circulation in acute myocardial infarctus, unstable angına[23], hyperlipidemia with a high cardiovascular risk[24,25], atherosclerotic peripheral artery disease[26] and coronary artery disease[27,28]. When Wang et al compared diabetes patients with OSAS and diabetes patients without OSAS, they stated that the possibility of complications due to arteriosclerosis and glycolipid metabolism disorder was higher[29]. A recent review of OSAS and cardiovascular disease risk emphasized that there is a positive association between OSAS and CVD risk, and the relative risk of CVDs continuously increases with the increase in AHI[30]. Talay et al demonstrated significantly higher PAPP-A levels in COPD patients than in a control group, and increasing PAPP-A levels could be a beneficial marker in the treatment of COPD, the aim of which is to prevent comorbidities such as adverse cardiovascular diseases[31]. Bulut et al reported that patients with asthma had significantly higher PAPP-A and IGFBP-4 levels than the control group. In the same study, it was reported that the PAPP-A level could be a useful biomarker for the prediction of airway remodelling in severe asthma patients who were taking omalizumab and that it could reflect response to treatment[32].

In the other study, it was shown that the serum PAPP-A level was elevated in lung cancer and it was emphasised that this increase in PAPP-A could be an inflammatory marker[33]. In contrast in another study, it was reported that there was no difference between PAPP-A levels in patients with pulmonary embolism and the control group, and

there was no correlation between PAPP-A levels and inflammatory markers[34]. A study by Cengiz et al was similar to the current study and high serum PAPP-A values were determined in OSAS patients compared to a control group. In that study, it was emphasized that there was a negative correlation between PAPP-A values and the AHI score. Decreasing PAPP-A levels in the severe OSAS group with AHI ≥ 30 were attributed to the compensatory mechanisms not having exceeded the oxidative and inflammatory activities[20]. Eickhoff et al also reported an association between the risk factors of OSAS such as hypoxia and chronic inflammation and vascular endothelial dysfunction and atherosclerosis[35]. While investigating the etiopathogenesis of OSAS, a relationship between OSAS and endothelial dysfunction was also found[36,37]. It has been explained that PAPP-A may be an important trigger of endothelial dysfunction and thus, PAPP-A regulates the production of Nitric Oxide and Endothelin -1, providing another important mechanism for its proatherogenic effect beyond lipid accumulation and vascular inflammation[38]. Based on this, although many mechanisms contribute to the endothelial dysfunction involved in the pathogenesis of OSAS, PAPP-A may contribute at this stage. In the current study, high serum PAPP-A levels were determined in the OSAS group compared to the control group, and as the severity of OSAS increased, so there was observed to be an increase in PAPP-A. Thus, unlike the findings of Cengiz et al, there was a positive correlation between the serum PAPP-A level and AHI score. Furthermore, although the PAPP-A level was not correlated with serum lipid levels, a relationship was determined with factors reflecting the metabolic profile such as insulin, HOMA-IR and BMI. These metabolic disorders can also be considered to be related to cardiovascular diseases. There was also determined to be a positive correlation between PAPP-A levels and average heart rate and oxygen desaturation index and a negative correlation with minimum oxygen saturation (%). This indicates the relationship between PAPP-A and hypoxia and insufficient oxygenation. Previous studies have emphasised that average heart rate is an independent predictor of coronary heart disease, stroke, sudden death and cardiovascular diseases[39]. From all these results, PAPP-A can be considered a predictor for cardivascular diseases.

Some limitations of this study should be considered, primarily the relatively low number of patients. Studies with larger samples would be useful to determine the reasons for high serum PAPP-A levels. A second limitation was that there was no evaluation of the examined parameters after treatment of the OSAS patients and therefore they could not be compared with the pre-treatment values. In addition, to be able to investigate the place of PAPP-A in the IGF system in OSAS pathophysiology, there could be examination of IGF parameters together with PAPP-A.

Conclusion

In conclusion, the results of this study demonstrated that PAPP-A levels were independently related to OSAS and OSAS severity. Thus for the first time, it can be stated that PAPP-A could be a clinical marker for increased risk of metabolic syndrome and cardiovascular events in OSAS. The use of PAPP-A, which provides prognostic information for the high risk of the development of cardiovascular disease in OSAS patients, could be of benefit in the evaluation of cardiovascular risk. There is need for further studies to confirm the predictive value of PAPP-A, and to determine the underlying mechanisms in OSAS patients. As more elevated PAPP-A levels were seen in severe OSAS, the proteins in this biochemical pathway warrant further investigation.

Conflict of interest

The authors declare that they have no conflict of interest.

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