J Appl Biomed
DOI: 10.32725/jab.2024.011
journal homepage: http://jab.zsf.jcu.cz

Journal of Applied Biomedicine

Original research article

The impact of obesity, age, and gender on plasmatic levels of selected glycoprotein biomarkers and miRNA-499 in OSA patients

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Abstract
Background: The current obstructive sleep apnea (OSA) diagnostic uses polysomnography or limited polygraphy and requires specialized personnel and technical equipment. Glycoprotein biomarkers and microRNAs are being explored as a possible new method for screening. We aimed to evaluate whether certain biomarkers and microRNA, previously identified as related to OSA, could be influenced by factors such as gender, age, and obesity level in patients with OSA.
Methods: In this retrospective analytical study, patients with moderate to severe OSA (n = 130) were compared with the control group. Serum levels of selected biomarkers and microRNA were taken from both groups. The group of OSA patients was then stratified by gender, obesity level, and age to see the possible influence of those variables on biomarker levels.
Results: Levels of all studied biomarkers – C-reactive protein (CRP), high-sensitivity troponin I (hsTnI), pentraxin-3 (PTX-3), and microRNA-499 were significantly higher in patients with OSA compared to the control group. In the OSA group only hsTnI showed a statistically significant relationship with gender. Levels of CRP and hsTnI showed a significant dependence on the level of obesity. Dependency on age was proven for hsTnI. CRP, PTX-3, and microRNA-499 did not have any statistically significant relationship with age.
Conclusion: We found that serum levels of pentraxin-3 and microRNA-499 in patients with moderate to severe obstructive sleep apnoea are independent of gender, obesity, and age. CRP was affected by the level of obesity and hsTnI was influenced by all 3 variables. We consider these findings important for further research of OSA biomarkers.

Keywords: Biomarkers; Diagnostic process; microRNA; Obstructive sleep apnoea

Highlights:
- The incidence of sleep apnea in the population is increasing.
- Scientific literature intensively researches the relationship between glycoprotein biomarkers, microRNAs, and sleep apnea.
- The study proves the influence of plasma levels of some biomarkers depending on obesity, age, and gender.
- These data are new, not yet published, and must be taken into account in subsequent research.

Abbreviations:
AHI, apnea-hypopnea index; BMI, body mass index; CPD, chronic pulmonary disease; CRP, C-reactive protein; DNA, deoxyribonucleic acid; DSIP, delta sleep-inducing peptide; hsTnI, high-sensitivity troponin I; ICAM-1, intercellular adhesion molecule 1; IRC, inter-run calibrator; miRNA, microRNA, micro-ribonucleic acid; ODI, oxygen desaturation index; OSA, obstructive sleep apnea; PCR, polymerase chain reaction; PG, polygraphy; PSG, polysomnography; PTX-3, pentraxin 3; RNA, ribonucleic acid; RT, reverse transcription; VCAM-1, vascular cell adhesion molecule 1

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http://doi.org/10.32725/jab.2024.011
Submitted: 2023-12-21 • Accepted: 2024-05-06 • Prepublished online: 2024-06-14
J Appl Biomed 22/2: 81–88 • EISSN 1214-0287 • ISSN 1214-021X

Introduction

The field of somnology, which focuses on the prevention and personalized treatment of sleep disorders, is a complex and demanding area that requires specialized personnel and equipment (Arachchige and Steier, 2022). Its primary goal is to improve patients' quality of life, personally and professionally (Jennum et al., 2014), by managing their basic disease and comorbidities (Pinto et al., 2016; Gottlieb and Punjabi, 2020). Cardiovascular diseases are the most frequent comorbidity of obstructive sleep apnoea (OSA). Pulmonary diseases (Wang et al., 2015), endocrine and hormonal pathologies (Akset et al., 2023), and metabolic diseases, especially diabetes mellitus, can also play a significant role in its development (Reutrakul and Mokhlesi, 2017; Xu et al., 2015).

The presence of apnoeic episodes can be proved by three basic diagnostic methods. Screening monitoring (only for SaO2, airflow in the airways, heart rate and snoring), limited polygraphy (PG), and polysomnography (PSG). Polysomnography and limited polygraphy provide sufficient data to diagnose the presence and type of OSA and are considered the gold standard for OSA diagnostics. The current diagnostic and monitoring scheme for OSA requires specialized equipment and expertise. One of the future ways for diagnostic process of OSA or monitoring the effectiveness of therapy will be the involvement of artificial intelligence (Verma et al., 2023), similar to that being explored in other fields of medicine (Filipovsky et al., 2023; He et al., 2021). Nonetheless, researchers are exploring alternative biomarkers to aid in the diagnosis and monitoring of OSA.

Several studies have investigated the relationship between OSA and biomarkers of inflammation (Imani et al., 2021; Sahlman et al., 2010), cardiovascular disease (Neumann et al., 2019), metabolism (Denver et al., 2011; Punjabi et al., 2002), oxidative stress (Katsoulis et al., 2011; Lavie, 2009), and adhesion molecules (Lv et al., 2021; Pak et al., 2015). While miRNAs, a group of markers that are easily accessible from peripheral blood sampling, relatively small in size (21–23 nucleotides), and exhibit good stability (Zapater et al., 2022), have shown promise in OSA research, research on their association with the disease is still in its early stages.

In this study, we aimed to evaluate whether certain glycoprotein biomarkers and microRNAs, previously identified as related to OSA, could be influenced by factors such as gender, age, and obesity level in patients who require positive airway pressure therapy.

Materials and methods

During a retrospective analytical study conducted in a tertiary hospital from the 1st of January 2015 to the 31st of December 2019, 169 patients who were suspected of having obstructive sleep apnea underwent sleep monitoring. After applying the exclusion criteria, 39 patients were excluded. The final sample size included 130 patients (details are provided in Fig. 1 flowchart).

Inclusion criteria: suspicion of obstructive sleep apnea, sleep monitoring done by polysomnography or limited polygraphy, apnea-hypopnea index (AHI) equal or more than 15 (moderate to severe obstructive sleep apnea), over 18 years of age.

Exclusion criteria: sleep monitoring other than limited polygraphy or polysomnography, any previous treatment for sleep apnea, upper airway surgery except adenotomy in childhood, a history of cerebrovascular disease, a history of cardiovascular disease (except hypertension), diabetes mellitus and other endocrinopathies, a history of cancer, chronic obstructive pulmonary disease, use of psychiatric medication, smoking and/or alcohol abuse, incomplete data, poor cooperation.

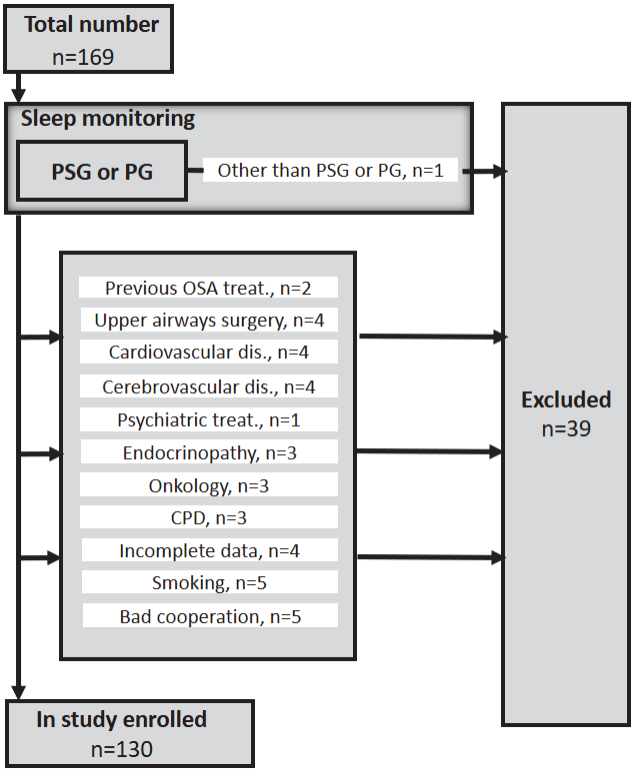

Fig. 1. Flowchart of the study

The study included a final cohort of 130 participants who represented the general population of sleep apnea patients. Of these, 48 were female (37.4%) and 82 were male (62.6%). The mean age of this cohort was 58.6 years, as shown in Table 1. The control group consisted of 81 healthy subjects who were selected from preventive examinations conducted by the staff of the University Hospital. Of these, 29 were women (35.8%) and 52 were men (64.2%). The mean age of the control group was 56.6 years. The absence of OSA in the healthy control group was determined based on the absence of symptoms and the Berlin questionnaire. The study cohort exhibited typical characteristics of patients with moderate to severe obstructive sleep apnea, with a mean BMI of 35.5 (median 34.4) and a mean AHI of 44.7 (median 40.9).

Sleep monitoring

Sleep monitoring was conducted using polysomnography (PSG) or limited polygraphy (PG). The classification of sleep apnea was done in accordance with the criteria set by the Czech Sleep Research and Sleep Medicine Society Website (Pretl et al., 2011). For this study, patients with an apnea-hypopnea index (AHI) of 15 or higher were included (refer to inclusion criteria). The other values that were measured during sleep monitoring included the desaturation index (ODI), arousal index, as well as the minimum and average nocturnal saturation. The assessment of sleep monitoring was carried out in accordance with the latest Czech Sleep Research and Sleep Medicine Society Website (Pretl et al., 2011) and AASM (2016).

Table 1. Comparison of the demographic distribution of study respondents and controls

		n	%	Mean age	Standard deviation	Median	Min	Max	Lower quartile	Upper quartile	*p*
Study cohort	Women	48	37.4	60.49	9.98	63.27	36.82	79.24	53.38	66.32	0.2048
	Men	82	62.6	57.51	12.89	57.53	25.98	86.65	47.52	67.42	
	Total	130	100.0	58.63	11.93	59.7	25.98	86.56	49.84	67.42	
Control	Women	29	35.8	53.80	12.29	55.83	35.70	74.21	43.09	64.13	
	Men	52	64.2	58.03	10.10	58.89	36.13	80.39	53.31	64.67	
	Total	81	100.0	56.61	11.05	58.39	35.7	80.39	48.52	64.45	

Sampling and analysis of glycoprotein biomarkers and miRNAs

Peripheral blood was drawn using VACUETTE® Z Serum Sep tubes (Greiner Bio-One, Kremsmünster, Austria). Serum was separated in 3 hours by centrifugation at 1700 × g for 10 min, and samples were immediately aliquoted and frozen at –80 °C (24 h temperature monitoring). Serum samples were thawed only once, just prior to the analysis. Serum levels of CRP, PTX-3, hsTnI, and miR-499 were measured for each sample.

Glycoprotein biomarkers analysis

Serum levels of CRP were measured with a chemiluminescent assay using a Maglumi Instrument (Snibe Co., Shenzhen, China). Pentraxin-3 was measured with a Simple Plex assay using the Ella System (ProteinSimple, San Jose, CA, USA). hsTnI were measured with chemiluminescent ACCESS assays using a UniCel DxI 800 Instrument (Beckman Coulter, Brea, CA, USA).

MiR-499 analysis

Reverse transcription (RT) real-time polymerase chain reaction (PCR) was used to quantify plasma miR-499 levels. The miRNA fraction of total RNA was manually extracted from 200 µl of blood plasma using the miRNeasy® Serum/Plasma Kit (Qiagen, Hilden, Germany). Additionally, 3.5 µl of 1.6 × 10^8 copies/µl of cel-miR-39 working solution (Qiagen, Hilden, Germany) was mixed in as an exogenous spike-in control to monitor extraction efficiency. The analysis only targeted mature miRNAs, not their precursors. Quantitative estimation of the selected miRNAs was performed by RT real-time PCR. TaqMan MicroRNA Reverse Transcription kit (Thermo Fisher Scientific, Foster City, CA, USA) was used for the RT reaction to prepare the cDNA template, followed by cDNA quantification by PCR using TaqMan miRNA Assays (Thermo Fisher Scientific, Foster City, CA, USA) in technical duplicates on a LightCycler® 96 system (Roche, Basel, Switzerland). The thermal profile of the PCR followed the manufacturer's protocol. To avoid inter-plate variation, inter-run calibrators (IRCs) were used (Hellemans et al., 2007). The deltaCt approach was used to calculate plasma miRNA levels of interest, which are presented as relative expression values calculated as 2 – (Ct of the miRNA of interest – Ct of the normalizer). Cel-miR-39 (exogenous reference) was used as the normalizer (Kroh et al., 2010; Pešta et al., 2019; Schwarzenbach et al., 2015).

Statistical evaluation, used analysis

SAS software (SAS Institute Inc., Cary, NC, USA) was used to perform statistical analysis. The measured parameters were used to calculate basic statistics such as mean, standard deviation, variance, median, interquartile range, minimum, and maximum. For categorical variables, absolute and relative frequencies were examined. Non-parametric tests, such as Wilcoxon Two-Sample Test and Kruskal–Wallis test were used to compare the distribution of the variables between the tested groups. The influence of OSA parameters and selected clinical data on the biomarker levels was investigated using multivariate regression. Relationships between variables were examined using correlation coefficients (Pearson correlation coefficient), and selected relationships were described using linear regression. The statistical significance was set at alpha = 5%.

Ethics

The Ethics Committee of the University Hospital in Pilsen approved the study (approval number: 130708), and all patients signed an informed consent form before their inclusion in the study.

Results

Plasma levels of studied markers

We observed a significant difference in serum levels of C-reactive protein, high-sensitivity troponin I, and PTX-3 among patients ($p < 0.0001$). The significance level for miRNA was $p = 0.0442$. For more information, please refer to Table 2.

Markers dependence on gender

Participants were categorized by gender; 82 males (62.6%) and 48 females (37.4%). The results only showed a statistically significant relationship for high-sensitivity troponin I ($p < 0.0001$) when comparing serum levels with gender. Please refer to Table 3 for further details.

Markers dependence on obesity (BMI)

The study group was analysed based on the level of obesity and categorized into four groups: those with a BMI of up to 24.9 ($n = 5$), those with a BMI of 25–29.9 ($n = 20$), those with a BMI of 30–39.9 ($n = 76$), and those with a BMI of 40 or more ($n = 30$). We measured the serum levels of the variables for all four groups and found that there was a statistically significant relationship between BMI and CRP and hsTnI ($p = 0.0002$ and $p = 0.0416$, respectively). However, Pentraxin-3 and miRNA-499 were not statistically related to BMI groups ($p = 0.1653$ and $p = 0.9976$, respectively). For more information, please refer to Table 4.

Table 2. Comparison of studied biomarkers plasma levels

Biomarker	Group	Mean	Standard deviation	Median	Min	Max	Lower quartile	Upper quartile	*p*
CRP (mg/l)	OSA	6.63	10.91	3.52	0.19	100	1.35	7.40	**<0.0001**
	Controls	2.434	3.397	1.192	0.145	22.92	0.563	3.127	
PTX-3 (pg/ml)	OSA	5628	2703	5240	588	12776	3669	7058	**<0.0001**
	Controls	3390	2119	2780	861	9409	1746	4533	
hsTnl (ng/l)	OSA	5.63	7.41	3.50	0.70	51.1	2.20	6.30	**<0.0001**
	Controls	3.548	4.843	2.4	0	37.3	1.5	3.4	
miRNA-499 (cycles)	OSA	0.0078	0.0206	0.0002	0	0.1312	0	0.0027	**0.0442**
	Controls	0.00003	0.000213	0	0	0.0015	0	0	

Table 3. Monitored variables dependence on gender

Biomarker	Gender	Mean	Standard deviation	Median (Min–Max)	Min	Max	Lower quartile	Upper quartile	Wilcoxon test *p*-value
CRP (mg/l)	Women	6.730	7.239	4.035	0.3	31.7	1.71	8.61	0.1322
	Men	6.584	12.651	3.228	0.19	100	1.205	6.327	
PTX-3 (pg/ml)	Women	5645	2468	5240	588	12686	4157	6762	0.7776
	Men	5617	2849	5242	940	12776	3569	7058	
hsTnl (ng/l)	Women	3.346	3.089	2.4	0.7	18.5	1.6	4.0	**<0.0001**
	Men	6.973	8.771	4.35	0.7	51.1	3.0	7.1	
miRNA-499 (cycles)	Women	0.00506	0.0113	0.0001	0	0.0587	0	0.003	0.5326
	Men	0.00936	0.0245	0.0003	0	0.1312	0	0.0023	

Table 4. Monitored variables dependence on BMI

Biomarker	BMI	Mean	Standard deviation	Median	Min	Max	Lower quartile	Upper quartile	*p*
CRP (mg/l)	Up to 24.9 (*n* = 5)	0.9268	1.274	0.462	0.28	3.198	0.3	0.466	**0.0002**
	25–29.9 (*n* = 20)	5.847	6.196	3.703	0.431	23.22	1.584	8.026	
	30–39.9 (*n* = 75)	6.094	12.628	2.653	0.19	100	1.239	4.906	
	40 and more (*n* = 30)	9.498	8.983	7.090	0.477	32.44	3.522	10.15	
PTX-3 (pg/ml)	Up to 24.9 (*n* = 5)	5986	3121	4474	2517	9920	4433	8585	0.1653
	25–29.9 (*n* = 20)	4551	2409	4310	588	9734	2853	5928	
	30–39.9 (*n* = 75)	5594	2625	5161	1714	12686	3638	6884	
	40 and more (*n* = 30)	6371	2896	6017	940	12776	4567	8529	
hsTnl (ng/l)	Up to 24.9 (*n* = 5)	3.66	2.159	3.7	1.0	6.6	2.3	4.7	**0.0416**
	25–29.9 (*n* = 20)	5.065	10.66	2.55	0.7	49.9	1.55	3.8	
	30–39.9 (*n* = 75)	5.432	7.117	3.5	0.7	51.1	2.2	6.1	
	40 and more (*n* = 30)	6.847	6.097	5.05	1.2	25.2	2.5	8.2	
miRNA-499 (cycles)	Up to 24.9 (*n* = 5)	0.0178	0.0391	0.0002	0	0.0788	0	0.001	0.9976
	25–29.9 (*n* = 20)	0.0057	0.0146	0.0001	0	0.0587	0	0.0018	
	30–39.9 (*n* = 75)	0.0077	0.0229	0.0004	0	0.1312	0	0.0025	
	40 and more (*n* = 30)	0.0072	0.0137	0.0002	0	0.06	0	0.0087	

Markers dependence on age

Neither the pentraxin group (CRP, PTX-3) nor miRNA-499 had any statistically significant relationship with age ($p = 0.6961$, $p = 0.2649$ and $p = 0.2504$, respectively). High-sensitivity troponin I had a statistically significant relationship with age in the study group ($p = 0.0004$). For details see Table 5.

Table 5. Monitored variables dependence on age

Biomarker	Pearson correlation coefficient	*p*
CRP (mg/l)	0.03445	0.6961
PTX-3 (pg/ml)	–0.09810	0.2649
hsTnl (ng/l)	0.30707	**0.0004**
miRNA-499 (cycles)	0.06865	0.2504

Discussion

The prevalence of obstructive sleep apnea (OSA) is increasing in both men and women, as documented in a study by Benjafield et al. (2019). The study compared data from 16 different countries (see Fig. 2). OSA is not only a severe disease from a medical perspective; it also places a significant financial burden on healthcare budgets, making it a significant socioeconomic problem (Potts et al., 2013).

This work investigates the applicability of various molecules, such as glycoproteins (CRP, pentraxin-3, hsTnI) and microRNAs (miRNA-499), in the diagnosis or monitoring of OSA. These molecules can be detected in peripheral blood sampling. The relationship between glycoproteins and OSA has been known for some time (Cahan et al., 1990; McKeon et al., 1990), but it has not been studied sufficiently. The role of selected miRNAs in the diagnosis of OSA is still in its early stages (Pinilla et al., 2021).

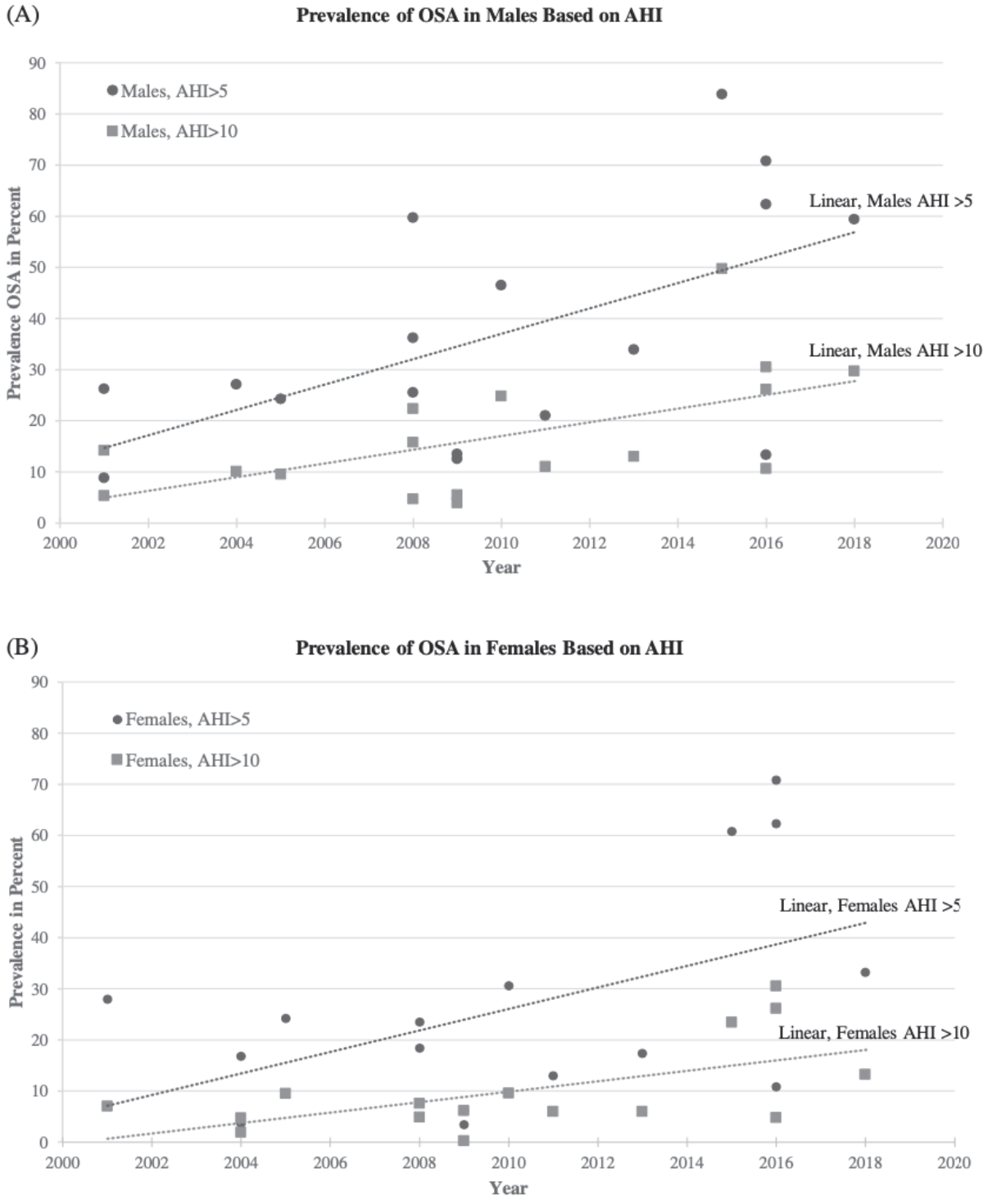

Fig. 2. Prevalence of OSA in males (**A**) and females (**B**) in 16 different countries (Benjafield et al., 2019; Lyons et al., 2020)

The study consisted of two groups of participants: patients with obstructive sleep apnea and a control group of healthy individuals. The patient group comprised 130 individuals diagnosed with moderate to severe obstructive sleep apnea, including 48 women (37.4%) and 82 men (62.6%), with an average age of 58.6 years. The control group included 81 individuals with an average age of 56.6 years, with 29 women (35.8%) and 52 men (64.2%). The two groups were comparable in age (p = 0.2048) and gender (p = 0.1443), as shown in Table 1. The control group was screened for any potential factors that could affect the results, such as diseases or the presence of obstructive sleep apnea, through routine examinations during preventive check-ups at the University Hospital in Pilsen and the results of the Berlin questionnaire (Solecká et al., 2022).

Studies conducted by Kanbay et al. (2015), Kobukai et al. (2014), Slouka et al. (2019), Sánchez-De-La-Torre et al. (2015), and our present study have demonstrated the correlation between OSA and the markers CRP, PTX-3, hsTnI, and miRNA-499. We also found a statistically significant relationship for all the markers studied, with p-values less than 0.0001 for CRP, PTX-3, and hsTnI, and p = 0.0442 for miRNA-499. Our investigation aimed to determine whether the study population's gender, age, and obesity variables could have influenced the results for these markers.

Our examination of gender dependencies did not reveal any significant influence for CRP (p = 0.1322), PTX-3 (p = 0.776), and miRNA-499 (p = 0.5326). These findings are consistent with those of Kelch et al. (2017) and Meder et al. (2014). Zapater et al. (2022) addressed gender differences for miRNAs in the OSA-targeted population, but their work investigated microRNA molecules different from ours. It should also be noted that there is still a lack of research on this topic. In contrast to the results above, we found that the levels of hsTnI were influenced by gender in our work. Males had higher plasma hsTnI levels than females (mean 6.97 vs. 3.35, $p < 0.0001$). To date, there are different data on the role of gender levels of hsTnI, but most studies are consistent in their conclusion that female physiological levels of high sensitivity troponin I are lower than male (Chaulin, 2023; Lee et al., 2019).

Our findings show that CRP (p = 0.6961), PTX-3 (p = 0.2649), and miRNA-499 (p = 0.2504) are not affected by age. However, hsTnI values were found to increase with age and this effect was statistically significant (p = 0.0004). The study conducted by Sánchez-de-la-Torre et al. in 2018 did not reveal any independent association between hsTnI and age in OSA patients. In contrast, the study conducted by Welsh et al. in 2018 showed an opposite trend of hsTnI levels; however, this study did not take into account troponin levels in OSA patients.

As expected, obesity (BMI) had an impact on plasma CRP levels (p = 0.0002), and hsTnI levels had a weak statistical relationship to obesity (p = 0.0416), with no clinical impact. Our results correlate with the findings of many studies (Choi et al., 2013; Hutchinson et al., 2000; Goulart et al., 2017; Rifai and Ridker, 2003). PTX-3 and miRNA-499 appeared to be independent of obesity (p = 0.1653 and p = 0.9976, respectively), confirming the findings of Kasai et al. (2011) for PTX-3. No similar data have been published for miRNA-499.

Research on biochemical biomarkers in the field of OSA has been ongoing for decades. As early as 1999, Ohga et al. studied oxidative stress, which causes an increase in the expression of adhesion molecules such as VCAM-1, ICAM-1, and L-selectin, among others. While miRNAs are a group of markers whose research concerning OSA is still underdeveloped, they have the advantage of being relatively small in size and having good stability. Recent scientific literature has papers dealing with the potential of microRNAs in the field of OSA. Zapater et al. (2022) published a paper highlighting the high potential of micro RNAs in this regard. Sánchez-de-la-Torre et al. (2015, 2018), co-authors of other publications agree that it is now possible to identify specific microRNAs associated with OSA and comorbidities. In particular, finding microRNAs that validate the cardiovascular risk associated with OSA would be beneficial. The team of Santamaria-Martos et al. (2019, 2020) published two papers confirming the above.

It is important to note that the presented study has some limitations. Firstly, the research was carried out at a single centre, which may affect the generalization of the results. Secondly, the number of research works on glycoproteins and their relation to obstructive sleep apnea (OSA) and microRNAs is quite low, but this is mainly because microRNA research is still in its early stages; until recently the tools to detect them were not widely available. However, due to the coronavirus pandemic, the equipment used for detecting microRNAs has rapidly expanded and is now available in most regional hospitals, including smaller ones. As a result, we can expect to see an increase in scientific data on this topic in the coming years.

Conclusion

In our study of patients with obstructive sleep apnea syndrome, we found that obesity had no impact on the serum levels of PTX-3 or microRNA-499. However, we did find a weak statistical relationship for hsTnI without clinical impact, and we did observe that obesity significantly affected CRP levels. Gender or age had no impact on the biomarkers CRP, PTX-3, and microRNA-499, but the biomarker hsTnI was influenced by these variables. These findings are crucial for biomarker and OSA researchers to consider.

Authors' contributions

Conceptualization, *DS, TK*, and *MP*; Methodology, *JW, DS*, and *BG*; Statistics and Methodology, *SK, MR*; Investigation, *JW, PŠ*, and *PK*; Writing – Original Draft Preparation, *DS, PK*, and *TK*; Writing – Review and Editing, *AS, VŠ*, and *RK*. All authors have read and agreed to the published version of the manuscript.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethical Committee of the University Hospital in Pilsen on 8 July 2013, approval no. 130708 of the Ethics Committee, University Hospital and Faculty of Medicine, Charles University, Pilsen. Informed consent has been obtained from the participants involved.

Funding

The work was supported by the Cooperatio Program, research area SURG, and by the Ministry of Health, Czech Republic – conceptual development of research organization (Faculty Hospital in Pilsen – FNPl, 00669806), and project BBMRI-CZ: Biobank network – a versatile platform for the research of the etiopathogenesis of diseases CZ.02.1.01/0.0/0.0/16 013/0001674, Bank of the clinical samples LM2018125.

Ethical aspects and conflict of interest

The authors have no conflict of interest to declare.

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