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Original Article

Evaluation of NPAS2 and Rev-Erb-α Serum Levels in Obstructive Sleep Apnea: Exploring Links Between Glucose Metabolism, Circadian Rhythm, and Hypoxia – A Pilot Study

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ABSTRACT

Obstructive sleep apnea (OSA) is recognized as a risk factor for type 2 diabetes mellitus (DM2). Since OSA disrupts circadian rhythms, it can influence circadian clock proteins such as neuronal PAS domain protein 2 (NPAS2) and nuclear receptor subfamily 1 group D member 1 (Rev-Erb-α). These proteins have been linked to metabolic disturbances, including insulin resistance. This pilot study aimed to examine serum levels of NPAS2 and Rev-Erb-α in patients with severe OSA and those with severe OSA combined with DM2, compared to healthy controls, and to assess their correlations with polysomnography (PSG) parameters, including oxygen saturation (SpO_2) measures. Forty participants were enrolled and divided into three groups: OSA ($n = 17$; AHI > 30, without DM2), OSA+DM2 ($n = 7$; AHI > 30 with DM2), and controls ($n = 16$; AHI < 5, without DM2). All participants underwent overnight PSG followed by morning blood sampling. Serum NPAS2 and Rev-Erb-α levels were quantified using enzyme-linked immunosorbent assay (ELISA). Mean NPAS2 levels were significantly lower in the OSA group compared to healthy controls ($p = 0.017$). The OSA group also showed lower NPAS2 levels than the OSA+DM2 group, though this difference only approached significance ($p = 0.094$). No significant differences were observed in Rev-Erb-α concentrations. A negative correlation was found between AHI during REM sleep and NPAS2 serum levels ($r = -0.478$; $p = 0.002$). NPAS2 serum levels may play a role in the metabolic dysregulation observed in OSA patients, potentially through mechanisms linked to REM sleep.

Keywords: Rev-Erb-α, Hypoxia, Circadian cycle, NPAS2, Glucose metabolism

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Introduction

Obstructive sleep apnea (OSA) is a sleep disorder characterized by repeated interruptions in breathing during sleep, resulting in intermittent hypoxia, frequent arousals, and fragmented sleep [1]. Polysomnography (PSG) is considered the diagnostic gold standard for OSA, providing a measure of disease severity via the apnea–hypopnea index (AHI) [2]. OSA is recognized as an independent risk factor for several metabolic disturbances, including impaired glucose regulation, insulin resistance, and type 2 diabetes mellitus (DM2) [3, 4]. These complications may be linked to imbalances in electrolytes and vitamins [5], disruptions in the serotonergic system [6–8], or changes in circulating adipokine levels [9].

Circadian clocks are intrinsic regulators of 24-hour cycles in physiological and behavioral processes [10]. Core circadian activator genes include CLOCK (circadian locomotor output cycles kaput) and BMAL1 (basic helix–loop–helix ARNT-like) [11]. These proteins bind to E-box elements to drive transcription of repressor genes such as period (Per) and cryptochrome (Cry) [12]. Neuronal PAS domain protein 2 (NPAS2), a member of the PAS transcription factor family, shares structural similarities with CLOCK and hypoxia-inducible factors, allowing it to substitute for CLOCK when necessary [13]. In mammalian circadian models, such as mice [14], CLOCK–BMAL1 activity peaks during daytime, promoting transcription of Per and Cry in the afternoon. PER and CRY proteins accumulate by evening, enter the nucleus at night, and inhibit CLOCK–BMAL1 activity, resetting the cycle for the following morning [15]. The circadian network also involves a feedback loop with nuclear receptor subfamily 1 group D member 1 (Rev-Erb-α), which interacts with CLOCK–BMAL1 to maintain positive and negative regulatory loops [16]. Dysregulation of this system has been associated with metabolic disturbances, including impaired glucose metabolism [17].

The aim of this pilot study was to assess serum levels of NPAS2 and Rev-Erb-α in patients with severe OSA, patients with severe OSA and DM2, and healthy controls, and to explore their associations with PSG-derived parameters.

Material and Methods

This cross-sectional investigation was conducted at the Department of Sleep Medicine and Metabolic Disorders, Medical University of Lodz, Poland, between January and June 2021. Eligibility criteria included age 18–70 years, BMI 20–40 kg/m², and written informed consent. Exclusion criteria were withdrawal of consent, chronic respiratory or inflammatory diseases, infection within one month of blood sampling, current or prior cancer, previously diagnosed sleep disorders aside from OSA, shift work, caffeine intake above 900 mg/day, and use of hypnotics or other sleep-influencing medications within two weeks of the study.

Patients referred with suspected OSA underwent a medical assessment and overnight PSG. DM2 status was determined from clinical history. Following PSG, venous blood samples were collected into serum separator tubes. Forty participants met inclusion criteria and were divided into three groups based on PSG and clinical data: OSA (severe OSA, AHI > 30, no DM2), OSA+DM2 (severe OSA, AHI > 30 with DM2), and control (no OSA, AHI < 5, no DM2). The study received approval from the Bioethical Committee of the Medical University of Lodz (RNN/432/18/KE).

Polysomnography

Participants were admitted to the sleep laboratory around 9:00 p.m. (±30 minutes). Standard physical and subjective assessments were performed, including measurements of height, weight, blood pressure, and heart rate. Overnight PSG included electroencephalography (EEG: C4–A1, C3–A2), electromyography (EMG) of the chin and tibialis anterior, electrooculography (EOG), oronasal airflow via thermistor, snoring, body position, respiratory movements of chest and abdomen (piezoelectric sensors), unipolar electrocardiography (ECG), and oxygen saturation (SpO_2) (Alice 6; Philips Respironics, Murrysville, USA). PSG events were scored by a single experienced physician, and sleep stages were classified in 30-second epochs according to standard criteria [2].

Biochemical analysis

Following overnight PSG, peripheral blood was drawn between 6:00 and 7:00 a.m., within 10 minutes of awakening, into tubes containing a clot activator or EDTA. Samples with a clot activator were centrifuged immediately at 4°C to separate the serum, which was then stored at −80°C. Serum concentrations of NPAS2 and Rev-Erb-α proteins were determined using an enzyme-linked immunosorbent assay (ELISA) kit (EIAaB, Wuhan, China), with absorbance measured at 450 nm using a BioTek 800 TS reader (Agilent Technologies Inc., Santa Clara, USA).

Statistical analysis

All statistical analyses were performed using Statistica 13 PL (StatSoft Polska, Krakow, Poland). Continuous variables were tested for normality using the Shapiro–Wilk test. Parametric data were compared with the Student's t-test, whereas nonparametric data were analyzed using the Mann–Whitney U test. Correlations were evaluated using Spearman's correlation coefficient. A p-value of less than 0.05 was considered statistically significant.

Results

Baseline characteristics of participants are presented in **Table 1**, and serum levels of NPAS2 and Rev-Erb-α are summarized in **Table 2**. Rev-Erb-α levels did not differ significantly between groups ($p= 0.624$). NPAS2 levels were significantly lower in the OSA group compared with healthy controls ($p= 0.017$). Although NPAS2 concentrations were lower in the OSA group than in the OSA+DM2 group, this difference did not reach statistical significance ($p= 0.094$), indicating only a trend. Among the analyzed correlations, a weak negative association was found between AHI during

REM sleep and NPAS2 serum levels ($r = -0.478$; $p = 0.002$).

Table 1. Baseline characteristics of the study population

Parameter		OSA group (n = 17)	OSA+DM2 group (n = 7)	Control group (n = 16)	p-value
Age [years] Me (IQR)		53 (44.50–59.50)	64 (56.00–72.00)	46 (33.75–56.50)	0.003*
Sex n (%)	M	14 (82.35)	6 (85.71)	11 (68.75)	0.548
	F	3 (17.65)	1 (14.29)	5 (31.25)	
BMI [kg/m^2] Me (IQR)		33.95 (30.99–37.54)	35.89 (32.08–42.67)	27.33 (24.27–28.88)	<0.001*
TST [h] Me (IQR)		6.50 (6.10–7.08)	5.46 (5.20–6.40)	6.20 (5.70–6.47)	0.050
Arousal index [events/h] Me (IQR)		23.70 (19.60–28.75)	28.10 (20.90–38.60)	12.25 (7.07–17.30)	<0.001*
AHI in REM sleep [events/h] Me (IQR)		38.97 (24.44–53.01)	47.51 (29.14–73.88)	1.64 (0.00–7.70)	<0.001*
AHI in NREM sleep [events/h] Me (IQR)		38.58 (32.33–61.15)	45.66 (35.69–62.89)	1.06 (0.35–1.64)	<0.001*
Total AHI [events/h] Me (IQR)		51.40 (35.9–64.15)	51.70 (45.70–63.40)	1.45 (0.52–3.00)	<0.001*
Desaturation index [events/h] Me (IQR)		50.0 (34.8–76.1)	60.0 (51.2–63.0)	2.0 (1.0–3.0)	<0.001*
SpO_2 during desaturation events [%] Me (IQR)		86.9 (80.5–90.1)	87.0 (83.8–88.0)	91.8 (90.5–93.1)	<0.001*

Me – median; IQR – interquartile range; OSA – obstructive sleep apnea; DM2 – diabetes mellitus type 2; M – male; F – female; BMI – body mass index; TST – total sleep time; AHI – apnea–hypopnea index; REM – rapid eye movement; NREM – non-rapid eye movement; SpO_2 – oxygen saturation; * statistically significant.

Table 2. Concentrations of circadian clock proteins

Parameter	OSA group (n = 17)	OSA+DM2 group (n = 7)	Control group (n = 16)	p-value
NPAS2 level [ng/mL]	117.07 ±55.29	198.28 ±259.83	186.22 ±166.31	0.037* 0.017*[a] 0.446[b] 0.094[c]
Rev-Erb-α level [ng/mL]	240.93 ±73.46	271.31 ±89.66	272.04 ±92.81	0.624 0.368[a] 0.947[b] 0.505[c]

Data presented as mean ± standard deviation (M ±SD).
NPAS2 – neuronal PAS domain protein 2; Rev-Erb-α – nuclear receptor subfamily 1 group D member 1; * statistically significant ([a] control group vs. OSA group, [b] control group vs. OSA+DM2 group, [c] OSA group vs. OSA+DM2 group).

Discussion

Disruptions in circadian clock gene function have been linked not only to sleep disorders, such as sleep phase syndromes [18], but also to metabolic disturbances, including metabolic syndrome [19] and insulin resistance [20]. In an experimental study, Zhang *et al.* observed that diabetic rats exhibited significantly elevated levels of Rev-Erb-α protein in adipose tissue compared with non-diabetic controls [21]. These findings suggest that Rev-Erb-α may play a central role in the development of metabolic abnormalities via circadian clock disruption [21]. Furthermore, Kooner *et al.*, analyzing a cohort of 5,561 individuals with type 2 diabetes, identified associations between NPAS2 gene variants and DM2 onset [22].

Research exploring circadian clock gene dysregulation in OSA patients remains limited, both in terms of publication volume and the number of genes investigated [23]. In a cross-sectional study of 49 participants, Canales *et al.* compared individuals with OSA or nocturnal hypoxemia (defined as ≥10% of total sleep time with $SpO_2 < 90\%$) to those without such conditions and found reduced mRNA expression of

Rev-Erb-α and NPAS2 in patients experiencing nocturnal hypoxemia [24]. However, since the study population consisted of patients with severe kidney dysfunction—a condition known to affect circadian gene expression [25]—it is difficult to determine whether the observed changes were attributable to OSA, hypoxia, or renal impairment.

Our pilot study aligns with findings by Xie *et al.*, who reported decreased NPAS2 gene expression in untreated OSA patients with an AHI > 15 [26]. Unlike their study, which did not reach statistical significance, our results showed a significant reduction in NPAS2 protein levels in the OSA group compared with healthy controls ($p = 0.017$). Future studies incorporating both NPAS2 gene expression and protein measurements, alongside CLOCK–BMAL1 assessments, could clarify the interactions among circadian activators in response to hypoxia during REM sleep.

Limitations

This study has several limitations, primarily the small sample size of 40 participants. The pilot design aimed to test feasibility and study methodology, which was successfully achieved, but larger cohorts will be needed for more robust conclusions. Variability in participant age and other characteristics made perfect matching challenging. Additionally, PSG recordings were conducted in a hospital sleep laboratory, which may have affected total sleep time due to unfamiliar surroundings and psychological adaptation, potentially confounding circadian-related sleep outcomes.

Conclusions

Serum NPAS2 protein may contribute to metabolic dysregulation observed in OSA patients, potentially through mechanisms related to REM sleep. Findings from this pilot study provide insight into the molecular pathways linking circadian rhythms, hypoxic stress, and glucose metabolism disturbances. However, further research is required to confirm the roles of NPAS2 and Rev-Erb-α in the development of type 2 diabetes mellitus.

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