Circadian melatonin secretion in obese adolescents with or without obstructive sleep apnea

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Abstract: Objective — To compare melatonin levels in saliva during a 24-hr day in order to identify the specificities of circadian melatonin secretion in obese adolescents with or without obstructive sleep apnea (OSA).

Material and Methods — We examined 18 obese adolescents with OSA, 12 obese adolescents without OSA, and 15 healthy adolescents with a normal body weight, from whom saliva was sampled four time during the 24-hr day. Polysomnography was used to diagnose OSA. Saliva samples (n=180) were subjected to enzyme-linked immunosorbent assay.

Results — Obese adolescents with OSA had higher evening melatonin levels than obese adolescents without OSA. For example, this indicator in OSA patients was 5.3 times higher than in participants without OSA, who had the lowest evening melatonin level among all groups. In both obese groups, nighttime melatonin levels were significantly lower than in the control group. A positive correlation was detected between the levels of morning and afternoon melatonin and body mass index only in obese adolescents without OSA (r=0.58; p=0.03 and r=0.68; p=0.01, respectively). It was found that evening melatonin correlated with minimum blood oxygen saturation (SaO2) in the entire sample of adolescents with OSA (r=0.69; p=0.008), and it also correlated with time with SaO2 <90% in the group with clinical manifestations of OSA (r=0.76; p=0.003). Nighttime melatonin levels negatively correlated with the minimum SaO2 value solely in the group with clinical manifestations of OSA (r=-0.58; p=0.035).

Conclusion — The circadian melatonin secretion in obese adolescents differed, depending on the presence or absence of OSA, and correlated with the level of oxygen desaturation in OSA patients, to a greater extent – in the presence of clinical manifestations.

Keywords: obstructive sleep apnea, obesity, melatonin, adolescents.

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Introduction

Obstructive sleep apnea (OSA) is a condition, characterized by recurrent episodes of upper airway obstruction and arousals, as well as by decreased oxygen saturation during sleep [1]. Repeated bouts of apnea/hypopnea, combined with awakening, result in sleep fragmentation and reduced sleep duration and quality. Some studies have shown a significant relationship between OSA and obesity [2-4]. These ailments lead to disruption of circadian rhythms and, as a consequence, to changes in the melatonin production during the 24-hr day, a decrease in the quality of life, etc. [5-9].

It is known that a large amount of melatonin is secreted by the pineal gland at night. Melatonin regulates a number of important central and peripheral processes associated with cyclic secretion. In addition, it can be important for slowing down the aging process, acting as an antioxidant, and can affect the regulation of the endocrine, reproductive, cardiovascular and immune systems, which could change in some pathological conditions [10, 11]. Exposure to artificial light source dramatically suppresses the release of melatonin at night. Studies on model animals and on humans have shown that melatonin secretion is closely related to sleep quality [12, 13]. Since OSA is known to alter the endogenous biological clock, and obesity may result from disorder of circadian rhythms, changes in melatonin secretion, simultaneously associated with OSA and obesity, are a very relevant topic for a study. However, there were no studies of circadian melatonin secretion in obese adolescents with OSA. Hence, the objective of our study was to reveal melatonin level dynamics in saliva over the 24-yr day in adolescents with or without OSA, in order to identify the specificities of circadian secretion of this hormone in obese subjects with or without OSA. We hypothesized that circadian melatonin secretion in obese patients would differ, depending on the presence or absence of OSA, and would correlate with some...
polysomnographic parameters, associated with OSA, in patients with simultaneous obesity and OSA.

Material and Methods

Study participants

The cross-sectional study design was applied to a survey of adolescents admitted to the Clinic at Scientific Centre for Family Health and Human Reproduction Problems (SC FHHRP) over the period from November 2017 through April 2018 due to obesity and other health conditions, a total of 45 male adolescents 15-17 years old. Participants with OAS (n=18) were recruited from the Somnology Center of SC FHHRP. The control groups with obesity (n=12) and normal body mass — NBM (n=15) included adolescents without episodes of snoring, OSA and with normal results of polysomnography (PSG) screening. It should be noted that OSA has sexual dimorphism [14], and men are especially vulnerable to OSA, so we included just boys in our study. All subjects underwent anthropometric measurements, questionnaires, PSG screening, and serial saliva sampling. The study was carried out over a single 24-hr day.

Inclusion criteria for the study were: a) boys 15-17 years old; b) body mass index (BMI) z-score >2 for corresponding age and gender for both obesity groups, or BMI z-score from -2 to +1 for the corresponding age and gender for the control group with NBM [15]; c) apnea/hypopnea index ≥2 for OSA [16]; d) signed voluntary informed consent. Exclusion criteria for study included the following: a) presence of sleep disorders other than OSA; b) taking exogenous melatonin, L-tryptophan, or sleeping draughts in two weeks preceding the study.

Measurements and procedures

We conducted questionnaires, anthropometric measurements, PSG, serial saliva sampling, melatonin level measurement, and statistical data processing.

Survey

The Adolescent Sleep Habits Survey (ASHS) was used to qualitatively assess habitual sleep and wakefulness, as well as its disturbances.

Anthropometric measurements

The anthropometric parameters (body mass and linear height) of youths were assessed, and BMI (kg/m²) was computed. Assessment of height and weight was carried out using weight-for-age reference curves sensu World Health Organization (WHO 2007) and the WHO AnthroPlus calculator. Weight status was determined by the value of the z-score [15].

Polysomnography

PSG screening was performed at the Somnology Center overnight using a stationary Grass-Telefactor TWin® PSG system (Comet) with an AS40 amplifier with a built-in SPM-1 sleep module (USA). PSG included recordings of: a) six leads of an electroencephalogram (EEG) (Fp3/Fp4, C3/C4, referring to O2/O1, respectively), b) electrooculogram (EOG), c) electromyogram (EMG) of submental muscles and tibialis anterior muscle, d) oronasal airflow pressure, e) snoring, f) thoracoabdominal breathing and g) blood oxygenation (saturation). The study was conducted in accordance with the recommendations of the American Academy of Sleep Medicine (AASM) [17]. Obstructive apnea was defined as a decrease in airflow of at least 90% for more than 10 seconds, and hypopnea was defined as a reduction in airflow of at least 30%, accompanied by a decline in oxygen saturation by 3%. The total number of apnea and hypopnea divided by the number of sleep hours was defined as the apnea and hypopnea index (AHI), the total number of EEG activations (arousals) per sleep hour was defined as the total activation index (AI).

Biomaterial sampling

Saliva samples for measuring melatonin levels were collected from each subject four times over 24-hr period: at 6-7 a.m., 12 noon-1 p.m., 6-7 p.m. and 11 p.m.-12 midnight [18]. To collect samples of biomaterial, special test tubes (SaliCaps, IBL International GmbH, Hamburg, Germany) were used, on which the subject’s code and time of collection were indicated beforehand. Study participants were instructed on how to collect and store samples prior to delivery to the research center.

Measuring melatonin level in saliva

The biomaterial was stored at -40°C until the analysis conducted at the laboratory. Melatonin levels (pg/mL) in saliva samples were measured by enzyme-linked immunosorbent assay (ELISA), using a commercial BÜHLMANN kit (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland) on ELx808™ Absorbance Microplate Reader (BioTek Instruments, Inc., VT, USA).

Statistical analysis

For statistical data processing, we used Statistica 10.0 software (StatSoft, Inc., USA).

The Kolmogorov-Smirnov test was employed to test the distribution (normal or abnormal) of the variables, and parametric or nonparametric methods were used, as needed. Data were expressed as frequencies or percentages, mean with standard deviation (M±SD), median (Me), lower quartile (LQ), upper quartile (UQ), minimum (Min), and maximum (Max). Comparisons of continuous variables among three groups (OSA group, obesity control group, NBM control group) were performed using one-way analysis of variance (ANOVA) or nonparametric Kruskal-Wallis H test (KW-H). The Pearson’s χ² test was used to compare participants for presence of ‘sleep problems’ versus ‘daytime problems’ (yes/no). The Spearman’s rank-order correlation method was used to analyze the relationship between melatonin levels at four time points vs. BMI depending on the presence or absence of OSA, as well as among melatonin content and PSG indicators, associated with OSA (AHI, minimum SaO₂, time with SaO₂ <90% and total AI), in all adolescents with obesity and OSA; and also among the levels of melatonin and aforementioned PSG indicators in groups with and without clinical manifestations of OSA.
Figure 1. Boxplot diagrams showing median, upper and lower quartiles, minimum and maximum salivary melatonin levels.

a) morning; b) noon; c) evening; d) night for each group of participants (1 – obese group with OSA; 2 – obese control group without OSA; 3 – control group with NBM). OSA, obstructive sleep apnea; NBM, normal body mass.
Table 1. Comparison of study groups by gender, age, BMI, ASHS scores and main PSG indicators

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Group with OSA (n=18)</th>
<th>Obese control group (n=12)</th>
<th>Control group with NBM (n=15)</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>1 vs. 2</td>
<td>1 vs. 3</td>
<td>2 vs. 3</td>
</tr>
<tr>
<td>Age, yr</td>
<td>16.2±0.3</td>
<td>16.1±0.2</td>
<td>16.0±0.7</td>
<td>0.819</td>
<td>0.542</td>
<td>0.726</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>2.5±0.2</td>
<td>2.4±0.1</td>
<td>-0.09±0.3</td>
<td>0.778</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASHS, n / %</td>
<td>10/55</td>
<td>10/83.4</td>
<td>14/93.3</td>
<td>0.114</td>
<td>0.016</td>
<td>0.412</td>
</tr>
<tr>
<td>Clinical</td>
<td>6/4/45</td>
<td>2/16.6</td>
<td>1/6.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Correlations between melatonin levels at four time points and OSA-associated PSG indicators in the general group of adolescents with OSA

<table>
<thead>
<tr>
<th>Indicators</th>
<th>AHI</th>
<th>Minimum So2O2</th>
<th>Time with So2O2 &lt; 90%</th>
<th>Total AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning melatonin level, pg/mL</td>
<td>-0.174 (p=0.489)</td>
<td>-0.211 (p=0.401)</td>
<td>0.377 (p=0.262)</td>
<td>-0.341 (p=0.206)</td>
</tr>
<tr>
<td>Daytime melatonin level, pg/mL</td>
<td>0.179 (p=0.477)</td>
<td>-0.089 (p=0.724)</td>
<td>0.147 (p=0.561)</td>
<td>0.056 (p=0.825)</td>
</tr>
<tr>
<td>Evening melatonin level, pg/mL</td>
<td>0.146 (p=0.562)</td>
<td>-0.541 (p=0.021)</td>
<td>0.192 (p=0.446)</td>
<td>-0.156 (p=0.536)</td>
</tr>
<tr>
<td>Nighttime melatonin level, pg/mL</td>
<td>-0.071 (p=0.779)</td>
<td>0.247 (p=0.323)</td>
<td>-0.036 (p=0.886)</td>
<td>-0.151 (p=0.548)</td>
</tr>
</tbody>
</table>

Table 3. Correlations between melatonin levels at four time points and OSA-associated PSG indicators in groups of adolescents with or without clinical manifestations of OSA

<table>
<thead>
<tr>
<th>Indicators</th>
<th>AHI</th>
<th>Minimum So2O2</th>
<th>Time with So2O2 &lt;90%</th>
<th>Total AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning melatonin, pg/mL</td>
<td>0.211 (p=0.558)</td>
<td>-0.063 (p=0.883)</td>
<td>-0.073 (p=0.841)</td>
<td>0.285 (p=0.494)</td>
</tr>
<tr>
<td>Daytime melatonin, pg/mL</td>
<td>0.017 (p=0.977)</td>
<td>0.192 (p=0.649)</td>
<td>-0.149 (p=0.681)</td>
<td>0.384 (p=0.347)</td>
</tr>
<tr>
<td>Evening melatonin, pg/mL</td>
<td>0.394 (p=0.261)</td>
<td>0.026 (p=0.952)</td>
<td>-0.584 (p=0.037)</td>
<td>-0.608 (p=0.027)</td>
</tr>
<tr>
<td>Nighttime melatonin, pg/mL</td>
<td>0.089 (p=0.808)</td>
<td>-0.304 (p=0.464)</td>
<td>0.081 (p=0.824)</td>
<td>-0.505 (p=0.042)</td>
</tr>
</tbody>
</table>

Results

Patient characteristics

Baseline characteristics, and ASHS and PSG data for the groups are presented in Table 1. Comparative analysis of the main characteristics showed that patients with obesity and OSA did not significantly differ in age from participants without OSA and from the control group with NBM. As expected, the BMI z-score was significantly different in non-obese adolescents compared with both obese groups (p<0.0001), but did not differ between patients with and without OSA. According to sleep self-evaluation, ‘sleep problems’ were noted by a much greater proportion of adolescents with OSA than in the control group with NBM, but not in the control group with obese subjects (p=0.016 and p=0.114, respectively). However, when comparing PSG indicators in the groups, statistically significant differences were revealed in superficial and deep sleep, sleep with rapid eye movements, sleep efficiency, AHI, time with SaO2 <90%, minimum So2O2 and AI in patients with OSA, relative to both control groups without OSA.

Salivary melatonin levels

A comparison of salivary melatonin levels during the 24-hr day between obese adolescents with OSA (1), obese subjects without OSA (2) and the control group with NBM (3) is shown in the Figure 1.

Obese patients with OSA had significantly higher evening melatonin levels (KW-H (2.45)=22.139, p<0.001) and a tendency towards higher night melatonin levels, compared with the obese control group. Obese patients without OSA had the lowest evening melatonin content among all groups of examined youths, which was statistically significantly lower than in the group with OSA, but
did not statistically differ from the values of the control group with NBM. Also, such patients tended to have higher morning melatonin levels (KW-H (2.45)=2.339, p=0.310) than their obese peers with OSA. Meanwhile, in both obese groups, nighttime melatonin levels were significantly lower than in the NBM control group (KW-H(2.45)=26.152, p<0.001).

Spearman’s rank-order correlation coefficient was calculated to assess the relationships between circadian melatonin secretion and BMI (depending on the presence or absence of OSA), as well as OSA-associated PSG indicators [AHI, minimum SaO₂ time with SaO₂<90% and total AI] – only in adolescents with obesity and OSA (Table 2).

Statistically significant positive correlations were found between BMI and melatonin levels in the morning and at noon only in obese adolescents without OSA (r=0.583, p=0.036; and r=0.682, p=0.012, respectively). The analysis of the relationships between melatonin secretion and OSA-associated PSG parameters showed that evening melatonin levels were significantly correlated with the minimum SaO₂ (r=-0.541; p=0.021). Other PSG parameters, associated with OSA, such as AHI, time with SaO₂ <90%, and total AI, did not correlate with daily dynamics of melatonin levels. Nighttime melatonin levels did not correlate with BMI or parameters, associated with OSA.

When compared correlation strength values between circadian melatonin secretion and OSA-associated PSG indicators in adolescents with and without clinical manifestations of OSA, no significant differences were found (Table 3), although evening melatonin levels negatively correlated with the minimum SaO₂ value, in adolescents with and without clinical manifestations of OSA (r=-0.608, p=0.027; r=-0.584, p=0.037, correspondingly). Moreover, the correlation analysis in the OSA group demonstrated a significant relationship between the concentration of nighttime melatonin content and minimum SaO₂ (r=-0.505; p=0.042), as well as between the evening melatonin levels and time with SaO₂ <90% (r=0.766; p=0.009), compared with non-OSA subjects.

Discussion

Our focus in this study was to examine the effect of OSA and/or obesity on circadian melatonin secretion in adolescents. We hypothesized that salivary melatonin levels over the 24-yr day in obese patients would differ, depending on the presence or absence of OSA, and would correlate with some OSA-associated PSG parameters.

It should be noted that significant differences in melatonin production over the 24-hr cycle could be the result of various ailments. In our study, we found that both obesity and OSA affect circadian melatonin secretion, which showed a trend towards higher evening melatonin levels in patients with OSA, as well as a tendency towards higher morning melatonin concentration in obese youths without OSA. However, unlike the control group of adolescents with NBM, the presence of a peak in nighttime melatonin secretion was not found in both obese groups. These results are similar to those in the study by Barnas et al. [19], conducted on middle-aged obese patients with OSA, which have found higher melatonin content during the day and lower melatonin concentration at night in these subjects than in the control group.

Meanwhile, Wikner et al. [20] showed that, in patients with OSA, neither the circadian rhythm of melatonin, nor the nocturnal melatonin concentration in blood serum, differ significantly from those in healthy people. After examining more recent publications on circadian rhythm of melatonin secretion in patients with OSA, we found that our results were at odds with the data of Wikner et al., as well as Brzecka et al. for adult patients with OSA. The authors found a tendency for an increase in melatonin concentration in the morning in 27% of patients with OSA, while only in 6% of the study subjects in the evening.

Hernandez et al. [7] and Zirkik et al. [21] described a morning serum melatonin peak at 6 a.m. in OSA patients. However, in individuals with nocturnal peak serum melatonin concentration, AHI was higher [5]. In contrast, we discovered that salivary melatonin level at night (11 p.m. to midnight) was significantly lower in obese patients (both OSA group and non-OSA groups) than in the NBM control group, and found a correlation between evening (6-7 p.m.) level of melatonin in saliva and the severity of desaturation in patients with OSA. The latter in our study did not correlate with the nighttime melatonin secretion.

It is known that the maximum melatonin production occurs in the dark. This hormone is central to chronobiology, being the main regulator of circadian rhythms. Impaired melatonin secretion may lead to disruption of the sleep-wake cycle [22, 23]. A deficiency in nighttime melatonin production in OSA patients, as well as increased evening melatonin levels, may explain daytime sleepiness and very rapid falling asleep (usually less than five minutes) in obese adolescents with clinical manifestations of OSA. However, nocturnal hypoxemia and repetitive arousal reactions are also major contributors to these changes. It is worth mentioning that the disrupted sleep-wake cycle is a key pattern in both obese patients with OSA and obese patients without it.

The change in the circadian melatonin production, found in obese adolescents in our study, is likely associated with causes other than OSA. This is not directly related to sleep because, unlike some studies [4, 24], we did not find any sleep problems in non-OSA obese adolescents. Melatonin provides regulation and synchronization of metabolic processes, has antioxidant and anti-inflammatory effects. A quantitative decrease in its level and/or a change in its secretion rhythm can lead to the development of various metabolic disorders, including obesity [25]. It is known that introduction of melatonin is the main element of therapy for both metabolic syndrome and obesity [26-28]. The Corbalan-Tutau’s study established that obese women had significantly reduced nighttime melatonin production [29], similar to our results for obese youths. This finding, along with a low level of melatonin in the evening, may indicate a change in the rhythm of this hormone production. Melatonin concentration normally begins to increase 1-2 hours before the usual time of going to sleep [30]. Surprisingly, non-OSA obese patients had higher melatonin levels at 6 a.m. than adolescents with OSA and control subjects with NBM.

Our results are more consistent with one of the first studies on the circadian melatonin rhythms in obese adolescents, which demonstrated a high average melatonin level but, at the same time, revealed a shift in the maximum melatonin secretion towards early morning [31]. Wetterberg et al. [32] found that nighttime melatonin concentrations negatively correlated with BMI. However, our results are at odds with those of Wetterberg, as we observed only significant positive correlations between morning and noon (but not nighttime) melatonin levels and BMI in the non-OSA group of obese patients. Perhaps, this finding indicates that, at the stage of functional (reversible) disorders, the activation of compensatory mechanisms occurs, an integral component of which is the
melatonin system, followed by depletion of reserve capabilities at the stage of somatic pathology formation.

No significant correlations were found between OSA-related PSG indicators and melatonin levels in the group of adolescents with no clinical manifestations of OSA, except for a positive correlation between the minimum SaO₂ level and evening melatonin content. Meanwhile, patients with clinical manifestations of OSA exhibited additional correlations between nocturnal melatonin and minimum SaO₂ levels, as well as between evening melatonin and time with SaO₂ <90%. Disruption of the sleep-wake cycle in OSA patients is connected to morning and afternoon sleepiness, along with mental and behavioral problems [33]. Sleep deprivation in adolescents also correlates with certain health problems [34]. In our study, more pronounced changes in circadian rhythms in adolescents with clinically significant OSA may explain the presence of the problems, associated with sleep and wakefulness.

Thus, we assume that, in conditions of obesity as an isolated pathology, there is a tendency to a shift in melatonin secretion towards the early morning, which indicates the participation of this hormone in the regulation of metabolic rhythms. When obesity is combined with OSA, the shift in melatonin secretion to the evening hours indicates the intensity of compensatory mechanisms that, in conjunction with some neurophysiological changes during sleep, may reflect an adaptive response to sleep fragmentation and intermittent nocturnal hypoxemia during episodes of apnea/hypopnea.

Conclusion

The results of our study indicated a change in circadian melatonin secretion of obese adolescents, both with and without OSA. However, in patients with clinical manifestations of OSA, more significant changes in melatonin production during the day were demonstrated, specifically, an increase in its level in the evening and production decrease at night. Our results emphasize the need to measure the circadian rhythm of melatonin secretion in obese adolescents with or without OSA which requires different approaches to treatment. New studies are needed to compare changes in circadian rhythm of melatonin production when different approaches are used to treat obese patients with or without OSA.

Limitations

The limitations of this study were related to the fact that small groups of adolescents were examined, given the incompetence of many potential participants who refused the upcoming PSG and saliva sampling, i.e. did not sign a voluntary informed consent. Hence, our results may differ from the results of studies with a larger sample size of obese patients.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical approval

The study was conducted in strict accordance with the Declaration of Helsinki (World Medical Association 2013), and the study protocol was approved by the Committee on Biomedical Ethics of SC FHHRP.

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