Salivary melatonin levels and sleep-wake rhythms in pregnant women with hypertensive and glucose metabolic disorders: A prospective analysis

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Summary In preeclampsia and gestational diabetes, the sympathetic nerves are activated, leading to disrupted sleep. Melatonin, which transmits information to regulate the sleep-wake rhythm and other such biorhythms, has been implicated in insulin resistance, antioxidant behaviors, and metabolic syndrome. In addition, its reduced secretion increases the risk of hypertension and diabetes. The aim of this study was to elucidate the features of melatonin secretion, sleep quality, and sleep-wake rhythms in pregnant women with complications. Fifty-eight pregnant women with pregnancy complications (hypertensive or glucose metabolic disorders) and 40 healthy pregnant women completed questionnaires, including sleep logs and the Pittsburgh Sleep Quality Index (PSQI), during the second to third trimesters. Their salivary melatonin levels were also measured. Pregnant women with complications had significantly lower morning ($p < 0.001$), daytime ($p < 0.01$), evening ($p < 0.001$), night ($p < 0.01$), daily mean ($p < 0.001$), peak ($p < 0.001$), and bottom ($p < 0.01$) melatonin values than healthy pregnant women. Pregnant women with complications also had significantly smaller melatonin amplitudes than healthy pregnant women ($p < 0.001$). Among pregnant women with complications, the duration ($p < 0.05$) and frequency ($p < 0.01$) of wake after sleep-onset were significantly greater in the poor sleep group than in the favorable sleep group which was divided by PSQI cutoff value. Pregnant women with hypertensive or glucose metabolic disorder complications had smaller circadian variation in salivary melatonin secretion, and their values were lower throughout the day than healthy pregnant women.

Keywords: Melatonin, preeclampsia, gestational hypertension, gestational diabetes mellitus, sleep-wake rhythm, sleep quality

1. Introduction

The incidence of pregnancy at an advanced maternal age is continuously increasing; however, it is associated with an increased risk of pregnancy-induced hypertension (1) and an increased incidence of gestational diabetes mellitus (GDM) (2). Under the conditions of preeclampsia (3,4) and GDM (5), the sympathetic nerves are activated. Moreover, patients with preeclampsia often experience sleep disruptions due to an increased frequency of movement during rapid eye movement sleep (6) and those with diabetes tend to have difficulty falling asleep (7).

Exposure to light at night due to an irregular sleep schedule increases the incidence of elevated blood pressure (8) and pregnancy complications (9); therefore, a regular sleep rhythm and quality of sleep are crucial issues during pregnancy. Melatonin, a neuroendocrine hormone secreted from the pineal gland (10), reaches maximum secretion levels 3-5 h after usual sleep-
onset (11) and decreases after awakening or upon light exposure (12). Melatonin is thus a humoral factor that transmits information to maintain biorhythms, such as the sleep-wake rhythm (13). Inhibited melatonin secretion in adult humans has also been associated with the incidence of obesity, metabolic syndrome (14), insulin resistance (15), and antioxidant activity (16). Experiments with rats have shown that melatonin stimulates insulin output and decreases free fatty acid synthesis (17). In addition, a large-scale survey of adult women demonstrated an association between low melatonin production and hypertension risk (18). Furthermore, the risk of developing diabetes has been reported to be higher among adults when melatonin secretion is low (19). However, no previous studies have assessed the association between melatonin secretion and the sleep-wake rhythm in pregnant women with certain complications. Thus, the aim of this study was to elucidate the features of melatonin secretion, sleep quality, and the sleep-wake rhythm in pregnant women with hypertensive or glucose metabolic disorders.

2. Methods

2.1. Subjects and diagnostic criteria

This study included 58 pregnant women who were diagnosed with a pregnancy-related complication (hypertensive or glucose metabolic disorder) in the second trimester (n = 35; week 16 day 0 to week 27 day 6) or third trimester (n = 23; week 28 day 0 to week 40 day 6). Patients were diagnosed using specified diagnostic criteria by a hospital obstetrician (20,21). All patients received prenatal checkups between February 2009 and April 2012 at a university hospital in the Saitama prefecture and provided informed consent to participate in the present study. Among the 35 women who were diagnosed in the second trimester and participated in the present study, 20 were continuously surveyed until the third trimester. Forty healthy pregnant women in the third trimester (week 28 day 0 to week 40 day 6), who received prenatal checkups between August 2006 and April 2008 at a hospital in Osaka Prefecture, were included as controls and each also provided informed consent to participate in the present study.

The diagnostic criteria for hypertensive disorders included conditions of preeclampsia, gestational hypertension, and chronic hypertension. Glucose metabolic disorders included diabetes mellitus and gestational diabetes mellitus (GDM), which was diagnosed according to the criteria revised in 2010.

2.2. Survey methods

Although the onset of pregnancy complications typically occur in the third trimester, the survey was initiated in the second trimester because severe cases develop from the second trimester and often result in delivery before the third trimester. Surveys were administered to the healthy control subjects in the third trimester. All participants who gave informed consent for the study filled out a self-administered questionnaire and took saliva samples themselves at home, and sent these to us by mail. They were required to complete a questionnaire describing their characteristics and lifestyles, the Pittsburgh Sleep Quality Index (PSQI), and sleep logs. Saliva samples were collected for measuring melatonin levels. Information on diagnoses and treatment courses were collected from medical records, as was medical information relating to the type of delivery, gestational weeks, birth weight, delivery abnormalities, and postpartum course.

2.3. Sleep logs

Sleep logs are used as a form of auxiliary diagnosis in the field of psychiatry and enable ascertainment of life rhythms (22). In the present study, sleep logs, in which the subjects filled in their sleep time every 30 min, were recorded daily for 1 week. Using a sleep-wake rhythm analysis program for Windows Ver. 3.0 (IAC, Tokyo, Japan), we analyzed sleep parameters that included total sleep time (total sleeping hours per day), nocturnal sleep time (sleeping hours per night), the duration of wake after sleep-onset [waking hours in the night (WASO)], the frequency of WASO, longest sleep time (LST), and sleep-onset time for LST.

2.4. Pittsburgh Sleep Quality Index (PSQI)

PSQI is a self-administered questionnaire (Likert scale of 0 to 3) developed by the University of Pittsburgh, Department of Psychiatry (Pittsburg, PA, USA) for assessing sleep quality (23). A Japanese language edition of PSQI was created by Doi et al. (24). The following seven components were scored: sleep quality (C1), sleep latency (C2), sleep duration (C3), habitual sleep efficiency (C4, the proportion of hours spent asleep in bed), sleep disturbance (C5), the use of sleeping medication (C6), and daytime dysfunction (C7). These elements were scored from 0 to 3, and the scores were added together to calculate a total PSQI global score (PSQIG, 0–21). For all items, the higher the score, the more sleep was determined to have been disrupted. The cut-off point for the total score was 5.5 points (23,24). Patients with a PSQIG < 5.5 points were included in the favorable sleep group, and those with a PSQIG ≥ 5.5 points were included in the poor sleep group.

2.5. Measurement of melatonin concentration

Saliva was collected at home 4 times daily for 3 days: before breakfast (6-8 a.m.), lunch (11 a.m.-1 p.m.),
Sleep parameters in pregnant women

3.2. Circadian variation in salivary melatonin concentration

For both pregnant women with complications in either the second or third trimester and healthy pregnant women, melatonin levels were the highest at night, decreased throughout the early morning, and were the lowest during the day, demonstrating significant daily fluctuations ($p < 0.001$) (Figure 1). Pregnant women with complications had significantly lower morning ($p < 0.001$), daytime ($p < 0.01$), evening ($p < 0.001$), night-time ($p < 0.01$), daily mean ($p < 0.001$), peak ($p < 0.001$), and bottom ($p < 0.01$) melatonin levels than healthy pregnant women (Figure 1). The amplitude, or the difference between the highest and lowest melatonin values in one day, was significantly smaller for pregnant women with complications than that for healthy pregnant women ($p < 0.001$). Among pregnant women with complications, the times of peak ($p < 0.05$) and bottom ($p < 0.05$) melatonin levels were significantly later in the third trimester than those in the second trimester (Table 2).

3.3. Sleep parameters in pregnant women

There were no significant differences in the analyzed sleep parameters between pregnant women with complications and healthy pregnant women in the third trimester. However, pregnant women with complications had significantly later sleep-onset times ($p < 0.01$), shorter LST ($p < 0.05$) and bottom ($p < 0.05$) melatonin levels than healthy pregnant women ($p < 0.01$). Among pregnant women with complications, the times of peak ($p < 0.05$) and bottom ($p < 0.05$) melatonin levels were significantly later in the third trimester than those in the second trimester (Table 3).

3.4. Comparison of sleep indicators according to PSQI

Among the pregnant women with complications, 15 (42.9%) were in the poor sleep group (PSQIG score $\geq 5.5$) in the second trimester and 23 (53.5%) were in the poor sleep group in the third trimester, while 20 women (50.0%) in the control group were in the poor sleep group during the third trimester. No significant difference in the frequency of poor sleep group and the mean value by PSQI, was observed between pregnant women with complications and healthy pregnant woman in the third trimester. Among pregnant women with complications, the duration and frequency of WASO of the sleep logs were significantly greater in the PSQI poor sleep group than those in the favorable sleep quality group in the second trimester ($p < 0.05$ and $p < 0.01$, respectively). In the third trimester, sleep-onset times were significantly later in the poor sleep group than those in the favorable sleep quality group ($p < 0.05$).
Correlation between melatonin values and sleep indicators

PSQI sleep efficiency (C4) for healthy pregnant women in the third trimester showed a negative correlation with nighttime ($r = -0.388$, $p < 0.05$), peak ($r = -0.324$, $p < 0.05$), and amplitude ($r = -0.324$, $p < 0.05$) melatonin values. However, in pregnant women with complications, these correlations were not observed in either the second or third trimester.

4. Discussion

This is the first study to analyze long-term melatonin secretion, sleep-wake rhythms, and sleep quality in pregnant women with hypertensive or glucose metabolism disorders.
metabolic disorders from the second to third trimester.

We measured salivary melatonin levels as a physiological indicator of sleep. Serum melatonin levels fluctuate throughout the day and peak at night (27). In the present study, we measured melatonin using saliva samples rather than blood samples because the salivary melatonin assay measured biologically active melatonin, since only free melatonin passes through the parotid membrane. Moreover, the collection of saliva is easier and less invasive than blood sampling at home.

Our study first elucidated that pregnant women with hypertensive or glucose metabolic disorder complications had lower melatonin secretion throughout the day (morning, daytime, evening, and night-time) than healthy pregnant women in both the second and third trimesters. Similar results were obtained with daily mean, peak, and bottom melatonin levels, indicating that pregnant women with complications have inhibited melatonin secretion throughout the day by measuring circadian variation in salivary melatonin secretion four times a day.

Melatonin is a humoral factor that controls the circadian rhythm of living organisms over approximately 24 hour cycles including the sleep-wake cycle, with high levels of melatonin observed after entering sleep at night. Insulin concentration, which adjusts blood glucose levels and blood pressure varies during this 24-hour cycle, with low levels reached at night. There are three types of melatonin receptors, comprising MT1, MT2 and MT3 receptors. When MT2 receptors bind with melatonin, this results in adjustment to the biological clock. There are also melatonin receptors present in the beta cells of the pancreas. MT2 receptors in beta cells of the pancreas are involved in insulin secretion adjustment as they block cyclic adenosine monophosphate (cAMP), an important metabolism signal that controls insulin secretion, and induce the formation of cyclic guanosine monophosphate (cGMP) (28).

However, genetic mutation in the MT2 receptors prevents them from responding to melatonin, thereby blocking insulin release from beta cells in the pancreas and increasing risk for type 2 diabetes. This has been confirmed by genetic analysis of approximately 12,000 subjects (29). Blood pressure exhibits diurnal variation over a 24-hour cycle mainly due to autonomic nervous action. Various factors are related to hypertension. These are thought to include, in addition to excessive sodium intake and autonomic nervous irregularity (30), disturbance of the biological clock and decreased melatonin secretion (31). Melatonin, for which high levels are observed at night during sleep, causes vasodilation, suppression of the sympathetic nervous system and hypotension (32). Melatonin increases cytoplasmic calcium and nitric monoxide in endothelial cells, thereby increasing vasodilation lowering the serum norepinephrine level. Therefore, low levels

Table 2. Melatonin indicators

<table>
<thead>
<tr>
<th>Items</th>
<th>Second-trimester pregnant women with complications ((n = 35))</th>
<th>Third-trimester pregnant women with complications ((n = 43))</th>
<th>Third-trimester healthy pregnant women ((n = 40))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td></td>
</tr>
<tr>
<td>Daily mean melatonin (pg/mL)</td>
<td>8.4 (1.2)</td>
<td>10.2 (1.4)</td>
<td>23.8 (2.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Peak value (pg/mL)</td>
<td>20.1 (2.8)</td>
<td>22.5 (2.9)</td>
<td>46.4 (4.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Bottom value (pg/mL)</td>
<td>1.7 (0.4)</td>
<td>2.4 (0.5)</td>
<td>7.3 (1.3)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Amplitude (pg/mL)</td>
<td>18.4 (2.6)</td>
<td>20.0 (2.6)</td>
<td>39.2 (3.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Peak value time (o'clock)</td>
<td>24.2 (0.9)</td>
<td>27.0 (0.7)</td>
<td>25.6 (1.0)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Bottom value time (o'clock)</td>
<td>15.6 (0.7)</td>
<td>17.7 (0.6)</td>
<td>16.9 (0.7)</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

\(^\dagger\)Comparison between third-trimester pregnant women with complications and healthy pregnant women using the unpaired \(t\)-test. \(^\dagger\)Comparison between second- and third-trimester pregnant women with complications using the unpaired \(t\)-test. SE, standard error.

Table 3. Sleep log indicators

<table>
<thead>
<tr>
<th>Items</th>
<th>Second-trimester pregnant women with complications ((n = 35))</th>
<th>Third-trimester pregnant women with complications ((n = 43))</th>
<th>Third-trimester healthy pregnant women ((n = 40))</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td></td>
</tr>
<tr>
<td>Total sleep time (h)</td>
<td>8.1 (0.2)</td>
<td>7.6 (0.2)</td>
<td>NS</td>
<td>7.8 (0.2)</td>
</tr>
<tr>
<td>Nighttime sleep time (h)</td>
<td>7.2 (0.2)</td>
<td>6.5 (0.2)</td>
<td>&lt; 0.01</td>
<td>6.8 (0.2)</td>
</tr>
<tr>
<td>Duration of wake after sleep-onset (min)</td>
<td>20.4 (4.6)</td>
<td>25.2 (5.8)</td>
<td>NS</td>
<td>25.4 (6.9)</td>
</tr>
<tr>
<td>Frequency of WASO (times)</td>
<td>0.2 (0.0)</td>
<td>0.3 (0.1)</td>
<td>NS</td>
<td>0.3 (0.1)</td>
</tr>
<tr>
<td>Longest sleep time (h)</td>
<td>7.1 (0.2)</td>
<td>6.2 (0.2)</td>
<td>&lt; 0.01</td>
<td>6.6 (0.2)</td>
</tr>
<tr>
<td>Sleep-onset time for LST (o'clock)</td>
<td>23.7 (0.2)</td>
<td>24.5 (0.2)</td>
<td>&lt; 0.01</td>
<td>24.4 (0.2)</td>
</tr>
</tbody>
</table>

Comparison between the second- and third-trimester pregnant women with complications using the unpaired \(t\)-test. Comparison between the third-trimester pregnant women with complications and healthy pregnant women using the unpaired \(t\)-test. SE, standard error; WASO, waking hours after sleep onset; LST, longest sleep time; NS, not significant.
of melatonin may be an onset factor for pregnancy complications, which exhibit pathophysiology that is similar to type 2 diabetes and hypertension (33).

Melatonin reportedly protects cellular apoptosis in the placenta by acting as an antioxidant (34) and a free radical scavenger (35). Melatonin also inhibits the vasospasticity of the umbilical artery (36), and when administered, possibly prevents the risk of preeclampsia (37). These findings suggest that the risk of preeclampsia is higher when melatonin secretion is decreased. Meanwhile, when melatonin secretion is reduced, the action of insulin receptors is negatively affected in adults (38), thereby increasing insulin resistance in non-pregnant women (15). Therefore, there is an increased risk of diabetes when melatonin secretion is reduced, which presumably also increases the risk of gestational diabetes in pregnant women.

In the present study, we observed later times of peak and bottom melatonin values in the third trimester compared with those in the second trimester; melatonin secretion rhythm lagged as a result of the significant delay in third trimester sleep-onset times. The amplitude in salivary melatonin secretion was decreased in pregnant women with complications in the third trimester in the present study and was likely because melatonin decreases after awaking, upon light exposure (12), or when sleep is shallow (39), and the sleep state often becomes shallow by polysomnography in the third trimester (40).

Healthy pregnant women with greater sleep efficiency had longer sleep times at night and greater melatonin peak values and amplitude. However, this trend was not observed in pregnant women with complications, as this group demonstrated shorter nocturnal sleep times and LST in the third trimester. This may have been because the sympathetic nervous system is dominant in women with gestational diabetes and preeclampsia (4, 30), meaning that secretion of the sleep-inducing substance melatonin at night, when the parasympathetic nervous system becomes dominant, was lower in pregnant women with complications than in healthy pregnant women in this study. This meant that they had poorer quality sleep and that healthy sleep efficiency and nighttime sleeping were impaired. Poor sleep efficiency, a PSQI component, due to arousal during sleep or difficulty falling asleep resulted in decreased melatonin secretion. However, melatonin secretion in pregnant women has been reported to correlate with melatonin secretion in cord blood (41) and to impact the production of fetal melatonin receptors through the placental stage (42), suggesting that melatonin secretion in pregnant women impacts the sleep-wake rhythm in infants after birth.

Therefore, promoting ample melatonin secretion by adjusting the sleep-wake rhythm during pregnancy and improving sleep quality is important not only to prevent hypertensive and glucose metabolic disorders but also to develop the sleep-wake rhythm in the fetus. Enhancing melatonin secretion requires strategies to increase sleep efficiency. For example, we recommend the following to improve sleep efficiency: darkening the room early at night (12), bathing before bed because body temperature and melatonin secretion are related (43), going to the toilet before bed to reduce WASO caused by frequent urination (40), going to bed early to increase sleeping hours (44), and maintaining a regular daily life rhythm.

There were certain limitations to the present study that should be addressed. First, we could not clarify the features of melatonin secretion associated with each diagnosis (hypertension and diabetes) because of the small number of subjects. In addition, the present study did not include matched controls because the control group only included healthy pregnant women in the third trimester.

5. Conclusion

Pregnant women with hypertensive or glucose metabolic disorders had smaller circadian variation in salivary melatonin secretion, and their melatonin values were lower throughout the day than healthy pregnant women. Moreover, pregnant women with these complications exhibited shorter nocturnal sleep times and LST. Overall, the sleep quality of both pregnant women with complications and healthy pregnant women was worse compared with that of non-pregnant women.

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