

## การเปรียบเทียบระดับเมลาโทนิน คุณภาพในการนอนหลับ และความตื่นตัว ในการทำงานระหว่างพยาบาลที่ทำงานผลัดกลางคืนและกลางวัน

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## Comparison of Melatonin Levels, Sleep Quality and Alertness between Nurses Working Night Shift and Day Shift

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**หลักการและวัตถุประสงค์:** การสัมผัสแสงไฟในตอนกลางคืน โดยเฉพาะในผู้ที่ทำงานผลัดคืน ส่งผลให้การหลั่งเมลาโทนินในเวลากลางคืนลดลง นอกจากนี้ คุณภาพในการนอนที่ลดลงจากการทำงานผลัดกลางคืน ส่งผลให้เพิ่มอัตราการเกิดอุบัติเหตุระหว่างทำงาน แต่ความสัมพันธ์ของคุณภาพการนอนกับการหลั่งเมลาโทนินยังมีข้อมูลไม่มาก การศึกษานี้จึงศึกษาหาความสัมพันธ์ระหว่างระดับเมลาโทนิน คุณภาพในการนอนหลับ และความตื่นตัวในการทำงาน เปรียบเทียบระหว่างพยาบาลที่ทำงานผลัดกลางคืนและกลางวัน

**วิธีการศึกษา:** การศึกษานี้เป็นการศึกษาภาคตัดขวางในอาสาสมัครที่เป็นพยาบาลที่ทำงานผลัดกลางคืน (จำนวน 30 ราย) และทำงานผลัดกลางวัน (จำนวน 29 ราย) มาอย่างน้อย 5 ปี เก็บปัสสาวะของอาสาสมัครสะสมตลอดคืน และวิเคราะห์หาระดับเมแทบอไลต์ (metabolite) ของเมลาโทนินในปัสสาวะ คือ 6-sulphatoxymelatonin (aMT6s) ด้วยชุดทดสอบ enzyme-linked immunosorbent assay (ELISA) และหาคุณภาพในการนอนหลับโดยใช้แบบสอบถามคุณภาพการนอนหลับ Thai Pittsburgh Sleep Quality Index (T\_PSQI) รวมทั้งประเมินความตื่นตัวในการทำงาน โดยใช้แบบสอบถาม Thai Karolinska Sleepiness Scale (T\_KSS) จากนั้นวิเคราะห์ข้อมูลโดยใช้สถิติ Mann-Whitney U Test และ Spearman's rho-correlation

**Background and objective:** Exposure to artificial light at night, particularly in night shift workers, leads to suppression of natural nocturnal melatonin production. Poorer sleep quality from shiftwork results in increased accident rates during the work, but there is limited data on sleep quality and melatonin production. This study examined the association between melatonin levels, sleep quality and alertness comparing between nurses working night shift and day shift.

**Methods:** The study was a cross-sectional design. Volunteer nurses working night shift (n=30) and day shift (n=29) during the past five years were recruited. Overnight urine samples were collected and assayed for 6-sulphatoxymelatonin (aMT6s), a melatonin metabolite, by enzyme-linked immunosorbent assay (ELISA). Sleep quality using the validated Thai Pittsburgh Sleep Quality Index (T\_PSQI), and alertness using the Thai Karolinska Sleepiness Scale (T\_KSS) were evaluated. Statistical analysis was performed using Mann-Whitney U-Test and Spearman's rho-correlation.

**Results:** The average level of creatinine-adjusted aMT6s in night shift nurses was lower, but not statistically significant, than that of day shift nurses (29.6±32.5 vs. 32.8±58.8 ng/mg Cr; p=0.439). The night shift nurses also

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**ผลการศึกษา:** กลุ่มพยาบาลที่ทำงานผลัดกลางคืนมีระดับ creatinine-adjusted aMT6s เฉลี่ยต่ำกว่ากลุ่มที่ทำงานผลัดกลางวัน แต่ไม่มีนัยสำคัญทางสถิติ ( $29.6 \pm 32.5$  และ  $32.8 \pm 58.8$  ng/mg Cr;  $p=0.439$ ) นอกจากนี้พบว่ากลุ่มพยาบาลที่ทำงานผลัดกลางคืนมีคุณภาพในการนอนต่ำกว่ากลุ่มที่ทำงานผลัดกลางวัน (T\_PSQI เท่ากับ  $7.5 \pm 5.0$  vs.  $5.0 \pm 2.8$ ;  $p=0.001$ ) และความตื่นตัวในการทำงานที่เวลาก่อนเริ่มงาน (T\_KSS เท่ากับ  $4.0 \pm 6.0$  vs.  $3.0 \pm 2.0$ ;  $p=0.006$ ) และระหว่างทำงาน (T\_KSS เท่ากับ  $6.0 \pm 3.0$  vs.  $3.0 \pm 1.8$ ;  $p < 0.001$ ) น้อยกว่ากลุ่มที่ทำงานผลัดกลางวัน ทั้งนี้ไม่พบความสัมพันธ์ระหว่างระดับ creatinine-adjusted aMT6s กับคุณภาพในการนอนและความตื่นตัวในการทำงาน

**สรุป:** ผลการศึกษาพบว่าพยาบาลที่ทำงานผลัดกลางคืนมีคุณภาพในการนอนลดลง แต่ยังไม่พบการเปลี่ยนแปลงของระดับเมลาโทนินอย่างมีนัยสำคัญ

**คำสำคัญ:** 6-sulphatoxymelatonin, ผู้ที่ทำงานเป็นผลัด, คุณภาพในการนอนหลับ, ความตื่นตัว, พยาบาล

reported poorer sleep quality (T\_PSQI were  $7.5 \pm 5.0$  vs.  $5.0 \pm 2.8$ ;  $p=0.001$ ) and less alertness both before (T\_KSS were  $4.0 \pm 6.0$  vs.  $3.0 \pm 2.0$ ;  $p=0.006$ ) and during (T\_KSS were  $6.0 \pm 3.0$  vs.  $3.0 \pm 1.8$ ;  $p < 0.001$ ) working. There was no correlation between creatinine-adjusted aMT6s and sleep quality nor alertness.

**Conclusion:** The findings showed poorer sleep quality in nurses working night shift without a significant difference of melatonin levels.

**Keywords:** 6-sulphatoxymelatonin, shift work, sleep quality, alertness, nurses

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## Introduction

Melatonin (N-Acetyl-5-methoxytryptamine) is an indole neurohormone synthesized and secreted in response to light by the pineal gland in mammals to generate a biological clock that maintains the body's circadian rhythms<sup>1</sup>. Peak levels usually occur after the onset of darkness or between 2.00-4.00 a.m. ( $50-200$  pg/ml) then gradually decline thereafter with minimal levels at daytime ( $0-20$  pg/ml)<sup>2, 3</sup>. Melatonin appears to have antioxidant and free radical scavenging properties, and attribute to immunomodulator and oncostatic agents<sup>4-6</sup>. The nocturnal melatonin signal provides circadian rhythm of all the cells of the body. Alteration of the patterns and/or levels of melatonin secretion have been reported to affect with sleep disorders, jetlag, depression, stress, reproductive activities, some forms of cancer and immunological disorders<sup>7, 8</sup>.

Exposure to artificial light at night leads to suppressions of natural nocturnal melatonin production. This is common in shift workers, involving over 20% of the population in developed countries. The association

of shiftwork and various adverse health outcomes including increased cancer risk has been reported<sup>9-12</sup>. Previous studies showed that night shift workers reported poorer sleep quality and quality of life, less night-time alertness and performance in than those in day shift. This was result to increase occupational injury, illness, medication use<sup>13-15</sup>. Relationship between melatonin level and its effect on sleep quality is uncertain.

Therefore, this study aims to examine the association between nightly melatonin production, sleep quality and alertness in nurses working at night compared to those working day time. Such relationship could enable future arrangement to minimize potential adverse effects of shift workers.

## Methods

### Study population

The study population was volunteer nurses who were working at Srinagarind Hospital, Queen Sirikit Heart Center of The Northeast, Maharat Nakhon Ratchasima Hospital, and primary care unit of Khon Kaen Hospital,

Thailand. The study was approved by the Khon Kaen University and Maharat Nakhon Ratchasima Hospital Ethics committee. Written informed consents were obtained prior to the initiation of this study. A sample size of 30 participants for each group was calculated from the expected difference of Pittsburgh Sleep Quality Index (PSQI) scores of 2 and standard deviation of 2.29.

The study was a cross-sectional design. The volunteers were registered female Thai nurses of ages 25-45 years old. The night shift group included nurses working night shift at least 5-6 clinical shifts per month and at least 10 months per year during the previous five years. The night shift was classified as work started about 12.00 a.m. and ended at 8.00 a.m. The inclusion criteria for the day shift group were nurses who did not have night shift during the previous five years and normally slept during the period of the night at least five days per week.

Nurses who had or previously had cancer and/or renal disease were excluded. In addition, nurses who were pregnant/lactating, or taking medications affecting the metabolism or timing of melatonin production in the last 30 days were also excluded. The listed medications are aspirin, ibuprofen, alpha-1 adrenoceptor antagonist group, beta-adrenoceptor antagonist group, estradiol and testosterone, benzodiazepine, alpha-2 agonist group, oral contraceptives and chlorpromazine, norepinephrine reuptake inhibitor and monoamine oxidase-A inhibitor group and benserazide<sup>16</sup>.

#### Analysis of urine 6-sulphatoxymelatonin (melatonin metabolite) and urine creatinine

At night time, the production of melatonin from pineal gland usually begins in the evening and reaches a maximum level between 2 and 4 A.M., before being gradually declining in the morning<sup>2</sup>. Accordingly, overnight urine, collecting all voided urine during the night up until 7.00 A.M., was used in order to cover the full production of melatonin at night. The total volume of urine for each subject was measured and recorded and a 12 mL aliquot was stored at -20°C until assay. The samples were assayed for 6-sulphatoxymelatonin

(aMT6s), a melatonin metabolite, by an enzyme-linked immunosorbent assay (ELISA) kit (RE54031, Lot No. EMS166) obtained from Immuno-Biological Laboratories (IBL, Germany) according to the kit protocol. Sensitivity of the kit was 1 ng/mL and the intra-assay and inter-assay coefficients of variation were 5.8-204 ng/mL (5.2-12.2%) and 12.4-220 ng/mL (5.1-14.9%), respectively. Cross reactivity for this kit was reported as less than 0.002% for melatonin, and 6-hydroxymelatonin, <0.0005% for N-acetyl-L-hydroxytryptamine, and less than 0.0001% for all other likely interferences. Urine creatinine was determined by routine hospital diagnostic laboratory at Srinagarind hospital.

The main advantages of ELISA are high specificity and sensitivity and the assay has a short processing time. Given that the precision of the ELISA is comparable to that of radioactive immunoassay (RIA), the sensitivity is well within the range of urine samples, and the correlation with RIA is high, therefore it represents the method of choice for the direct measurement of aMT6s in urine<sup>17</sup>. In addition, the concentration of melatonin metabolite, aMT6s, in urine is 20-30 times higher than unmetabolized melatonin<sup>18</sup>. Measuring urinary aMT6s, is a noninvasive method and a good representation of melatonin in serum. Strong correlation of serum melatonin and urinary aMT6s ( $r=0.86$ ;  $p<0.0001$ ) was previously reported<sup>19</sup>. In this study the levels of aMT6s were also adjusted with urine creatinine in order to account for variations of urine volume which may lead to difference concentration of aMT6s, and to allow comparison to other studies.

The volunteers also completed questionnaires about their demographic data, health status, family history of cancer, working status, alcohol intake, smoking, exercise, children, and bed partner or roommate. Subjects were asked to refrain from the use of alcohol and caffeine intake; this was also reaffirmed and recorded on the date of urine collection.

#### Assessment of sleep quality

The validated Thai version of the Pittsburgh Sleep Quality Index (T\_PSQI) was used to measure sleep

quality<sup>14, 20</sup>. The PSQI is a self-rated questionnaire assessing sleep quality and disturbances over one month period. Nineteen individual items generate seven component scores, which are subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. Each component score has a range of 0-3 points. In all cases, a score of “0” indicates no difficulty, while a score of “3” indicates severe difficulty. The seven component scores are then added to yield one “global” score, with a range of 0-21 points: “0” indicating no difficulty and “21” indicating severe difficulties in all areas. Poor sleepers are people who have a global PSQI score greater than five. A global PSQI score greater than five was reported a diagnostic sensitivity of 89.6% and a specificity of 86.5% ( $\kappa=0.75$ ,  $p<0.001$ ) in distinguishing good and poor sleepers<sup>20</sup> and in insomnia patients versus controls<sup>21</sup>. Volunteers completed the questionnaire before their working time (11.45 p.m. for the night shift nurses and 7.45 a.m. for the day shift nurses) and returned them to the researchers.

#### Assessment of Alertness

The subjects also completed the single question Thai Karolinska Sleep Scale (T\_KSS; a 9-point scale ranging from “extremely alert” to “extremely sleepy—fighting sleep”)<sup>22</sup>. Thai version was translated from the English version and checked by an expert person. The T\_KSS score was recorded at three times: just before the subject started working at midpoint of the shift, and after working times (11.45 p.m., 4.00 a.m. and 7.15 a.m. for the night shift nurses and 7.45 a.m., 12.00 p.m. and 3.15 p.m. for the day shift nurses).

#### Statistical Analysis

Data analysis was performed using SPSS 15.0 for Windows. Statistical methods included comparisons of frequency (Mann-Whitney U-test) and correlation test (Spearman’s rho-correlation). Statistical significance was considered as  $p<0.05$ .

## Results

Demographic data of 59 nurses (night shift nurses=30 and control group=29) are presented in Table 1. The night shift group was significantly younger than the day shift (37 vs. 42;  $p=0.003$ ). Furthermore, parity ratio (had children) and sleeping partner ratio of the night shift nurses were also slightly lower than the day shift nurses (53% vs. 79%;  $p=0.035$  and 60% vs. 79%;  $p<0.001$  respectively). Night shift nurses had longer years of working than the day shift group (13 vs. 9 years;  $p=0.008$ ).

The average of creatinine-adjusted aMT6s level of the night shift nurses was lower than the day shift nurses, but not statistically significant different (median $\pm$ inter quartile range 29.6 $\pm$ 32.5 vs. 32.8 $\pm$ 58.8 ng/mg Cr;  $p=0.439$ ) (Figure 1). Following the guidance of a good sleeper having PSQI Global Score  $\leq 5$ , the sleep quality of day shift nurses was significant better than the night shift nurses (T\_PSQI Global Score 5.0 $\pm$ 2.8 vs. 7.5 $\pm$ 5.0;  $p=0.001$ ). In addition, good sleepers were accounted for 64.3% of the day shift nurses and only 20% in the night shift nurses were good sleepers. The night shift nurses also reported significantly less alertness, higher T\_KSS score, both before (4.0 $\pm$ 6.0 vs. 3.0 $\pm$ 2.0;  $p=0.006$ ) and during (6.0 $\pm$ 3.0 vs. 3.0 $\pm$ 1.8;  $p<0.001$ ) working than the day shift nurses, but not significantly different alertness after working (3.0 $\pm$ 3.3 vs. 4.0 $\pm$ 2.0;  $p=0.756$ ), from the KSS 9-point scale ranging from “extremely alert” to “extremely sleepy-fighting sleep” (Table 2). There were no significant correlation between creatinine-adjusted aMT6s and sleep quality, alertness, age, BMI and years of work (data not shown).

Table 1 Demographic data of the studied nurses

Characteristics	day shift nurses n=29	night shift nurses n=30	P-value
Age (year) <sup>†</sup>			
Median±IQR	42.0±4.5	37.0±10.3	0.003
Years of work (year) <sup>†</sup>			
Median±IQR	9.0±7.0	13.3±9.5	0.008
BMI <sup>†</sup>			
≤ 21	11 (37.9%)	18 (60%)	0.090
> 21	18 (62.1%)	12 (40%)	
History of disease <sup>†</sup>			
Yes	8 (27.6%)	12 (40.0%)	0.314
Family history of cancer <sup>†</sup>			
Yes	10 (34.5%)	9 (30.0%)	0.713
Exercise <sup>†</sup>			
No or once in a long while	16 (55.2%)	17 (56.7%)	0.692
≤ 3 time/week	8 (27.6%)	10 (33.3%)	
> 3 time/week	5 (17.2%)	3 (10.0%)	
Parity <sup>†</sup>			
Yes	23 (79.3%)	16 (53.3%)	0.035
Sleeping Partner <sup>†</sup>			
Yes	23 (79.3%)	18 (60.0%)	< 0.001

<sup>†</sup>Mann-Whitney U-test, <sup>†</sup>Chi Squared test

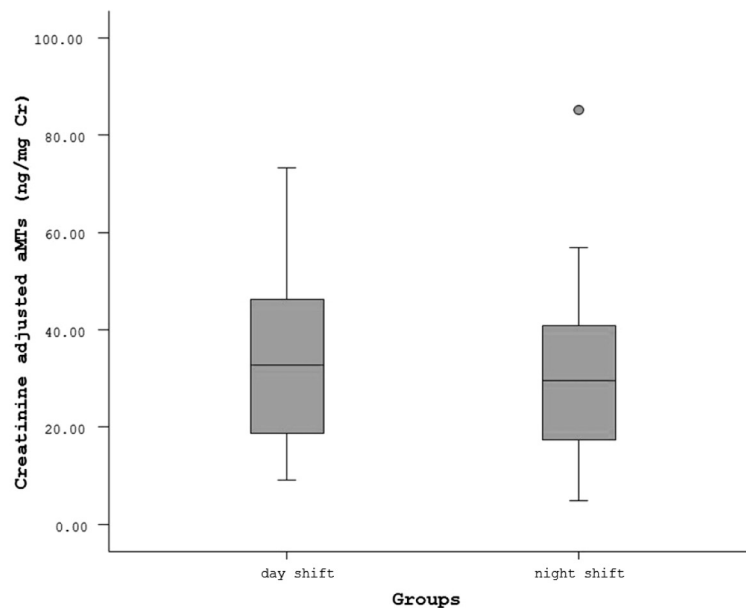


Figure 1 Box plot of Creatinine adjusted aMT6s (ng/mg Cr) between day shift nurses and night shift nurses

**Table 2** Median±IQR of aMT6s, urinary creatinine levels, creatinine adjusted aMT6s, T\_PSQI score and KSS score from 59 nurses, day shift (n=29) and night shift nurses (n=30)

Parameters	day shift nurses	night shift nurses	P-value*
	n=29	n=30	
	Median±IQR	Median±IQR	
aMT6s (ng/mL)	29.3±20.5	21.7±27.7	0.135
Creatinine (ng/mL)	98.3±123.1	71.5±68.7	0.126
Creatinine adjusted aMT6s (ng/mg Cr)	32.8±58.8	29.6±32.5	0.439
T_PSQI Global Score	5.0±2.8	7.5±5.0	0.001
T_PSQI Global Score>5 <sup>†</sup>	30.86±57.24 n=10 (35.7%)	21.82±30.16 n=24 (80%)	0.140
KSS Score <sup>‡</sup>			
Before working	3.0±2.0	4.0±6.0	0.006
During working	3.0±1.8	6.0±3.0	< 0.001
After working	4.0±2.0	3.0±3.3	0.756

\* Mann Whitney U-test

<sup>†</sup> Scores more than five means 'poor sleep'

<sup>‡</sup> Higher scores mean 'less alertness'

## Discussion

The study showed poorer sleep quality in nurses working night shift. There is a trend of decreased aMT6s, melatonin metabolite produced at night, but not significant, and no association with sleep was found. Many previous studies demonstrated a progressive decline in the amplitude of melatonin rhythm in the elderly, especially in subjects over 70 years<sup>1</sup>. Increasing age was related to decreasing in aMT6s production and excretion, both 24 hour and overnight<sup>23</sup>. Increased BMI could contribute to the decrease in melatonin levels observed in early childhood due to constant melatonin production with increasing size of the human body, and in 35-50 years old primarily premenopausal women<sup>18, 24, 25</sup>. Interestingly, our study found that night shift nurses were younger than those working day shift (median 37 vs. 42, p=0.003), but they reported a lower aMT6s level (29.6±32.5 vs. 32.8±58.8 ng/mg Cr; p=0.439) than day shift workers. Low levels of melatonin in younger nurses could be partly due to their exposure to light at night through working night shift.

Schernhammer et al., reported significantly lower melatonin levels in night shift workers, and those

working more than four nights in the two weeks before urine collection had lower levels than who never worked<sup>18</sup>. In our study night shift nurses exposed to light at night for at least 5-6 clinical shifts per month and at least 10 months per year during the previous five years also had lower melatonin levels. Therefore such duration of working night shift could lead to more melatonin reduction as seen in this study.

Other factors, such as BMI or underlying disease could also affect melatonin levels<sup>18, 26</sup>. However, such confounding factors were comparable in both groups. This study also controlled for drug-induced melatonin production, and this should not affect the results. However, we did not control for external sources of melatonin intake, such as food. For example, Kovacs et.al., measuring the level of melatonin after taking melatonin orally controlled caffeine and alcohol consumption one day before the test day<sup>19</sup>. These factors were not controlled in our study, however, this should not affect the results as previous study has shown that caffeine intake does not affect endogenous melatonin production<sup>16</sup>. In addition, vegetable intake was reported to associated with urinary aMT6s levels<sup>27</sup>. Melatonin can be found

in various medicinal and edible plants such as fever few, St. John's wort, seeds and fruits<sup>27, 28</sup>. As we did not control for food consumption factor in this study, it may interfere with the results and lead to high variation as shown in this study. Therefore, this should be taken into consideration when interpreting the results of this study.

The T\_PSQI is a well validated measure and was previously tested in a Thai population before use in the study<sup>14</sup>. T\_KSS, however, has never been tested before in a Thai population. The measure, comprising one single item with nine choices, was instead content validated by an expert prior to its use. In addition, the results from KSS test is highly correlated with T\_PSQI, ( $r=0.341$ ,  $p<0.009$ ). Therefore, the results of KSS from this study are considered valid.

Previous studies showed that nurses working night shift had lower sleep quality than the day shift (77% vs 51%), and resulted in more medical errors, occupational injuries and illness<sup>13,14</sup>. Our study showed similar results regarding poor sleep quality in night shift workers. However, no correlation between sleep quality and melatonin levels was shown in this study. This could be due to the small sample size with wider variation of melatonin level. As reduction of melatonin can lead to circadian rhythm disruption and is associated with adverse health outcomes in night shift workers<sup>(9-12)</sup>, further study to evaluate such associations should be conducted. Larger sample size, calculated from high variation of aMT6s level endpoint, using wider socioeconomic background of nurses, and controlling for potential food source of melatonin are warranted. This could enable future recommendations to minimize potential adverse effects to shift workers.

### Conclusion

The study showed poorer sleep quality in nurses working night shift. There was a trend of decreased aMT6s levels, the melatonin metabolite produced at night, but it was not statistically significant, and no association with sleep was found. The negative findings could be due to small sample size and various

demographic characteristics of the population. There is increasing concern that night shift work results in poor sleep quality and decreasing endogenous melatonin production at night, compromising protection against its natural oxidative properties. If the true association of melatonin and sleep was confirmed, supplementary melatonin may potentially minimize the risk of adverse health outcome in this population.

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