

A STUDY OF MELATONIN LEVELS AND STRESS IN FEMALE SHIFT WORKERS

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Abstract. The aim of this study was to determine the relationship between saliva melatonin and stress levels in Thai female shift workers. Five older (38.4 ± 1.82) and five younger (21.4 ± 0.55) female workers voluntarily participated in this study. All participants worked both morning and night shifts at a glass manufacturing factory. Saliva was collected every three hours at the workplace and at the subjects' houses to examine melatonin profiles. The Mann-Whitney *U* test and the Wilcoxon signed ranks test were used. There was a significant ($p < 0.05$) difference between melatonin levels in younger and older subjects when measured during the night shift at 19:00. Differences between melatonin levels during the morning and night shifts in the older group were significant at 07:00 and at 19:00 in younger subjects ($p < 0.05$). Normal stress and mild stress were found. No significant differences in melatonin levels were found between workers with normal and mild stress levels. The onset time of increasing saliva melatonin was at 19:00, both in women working the morning shift and in those working the night shift. Peak melatonin production occurred at 22:00 for the night shift in both groups. During the morning shifts, the peak times were at 04:00 and 01:00 (in the younger and older groups, respectively), usually between 02:00 and 04:00. These findings show that melatonin levels in female shift workers adapted according to the shift worked, especially in the older group. Health surveillance programs should therefore be established to prevent further negative health effects for female shift workers.

INTRODUCTION

In Thailand, 71% of businesses have a shift work system in which 92% of shift workers are working fixed shifts (Mouri and Phuntujaroensri, 1998). Increased manufacturing demand causes laborers to work extended hours. Long working hours can disrupt the circadian rhythms of shift workers (Minors and Waterhouse, 1981; Waterhouse *et al*, 1989; Baxter *et al*, 2000; Rajatnam and Arendt,

2001), an effect known as "shift lag syndrome". Shift workers who have shift lag syndrome make more mistakes and have an increased incidence of injuries and accidents during work (Giovanni, 1996; Baxter *et al*, 2000). Older and married shift workers are at risk for physiologic disturbances due to family responsibilities, time of sleep and unscheduled work, especially in older female workers (Roman, 1997).

Shift work and working extended hours (over 40 hours/week) are unnatural behaviors, and are indirect causes of stress. Stress, a subjective syndrome that is difficult to define, is the most important factor influencing quality of work performance. Shift work does not directly cause stress or strain but affects the functioning of hormones, such as cortisol and

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melatonin (Monk and Folkard, 1992; Baxter *et al*, 2000). Melatonin is a marker of circadian rhythm, as are cortisol secretion, core temperature and prolactin (Rajatnam and Arendt, 2001). There are many situations in which it would be useful to know melatonin levels in order to determine the phase state of the biological clock (Rohr and Herold, 2002). Because shift work leads to stress and impairment in health, this study used melatonin profiles to investigate the health effects of shift work.

MATERIALS AND METHODS

Subjects

Ten female shift workers in a glass factory located in Samut Prakarn, Thailand participated in this study. Subjects were randomly selected to control external variables (type of work, work duration, break times). Their mean ages were 21.4 (range 20-25) years for the younger group and 38.4 (range 35-40) years in the older group. All subjects were interviewed and excluded if they were sick, regularly consumed alcohol, smoked, or had a sleep disorder. Subjects changed shifts every two days, working two morning shifts, two afternoon shifts, and then two night shifts. The study was conducted between 13-18 June, 2002.

Experimental protocol

All subjects were working in the same job and shift schedule in the glass manufacturing plant. Food intake was controlled because it is a strong confounder. Workers were not allowed to eat any food within 30 minutes before saliva samples were taken, and were instructed to avoid coffee, cocoa, bananas, and beverages containing artificial colorants or alcohol. If sampling was scheduled to occur during an eating period, subjects were requested to postpone their meal, were permitted to eat immediately after the collection, and asked to rinse their mouths with water 15 min-

utes before the next collection. Certain environmental data, including light intensity and temperatures for the WBGT heat stress index, were measured near the subjects' working areas. General environmental data were also recorded.

Questionnaires

Subjects were interviewed to obtain general information, health status and sleep quality on 13 June, 2002. The sleep quality section was developed from the Pittsburgh Sleep Quality Index (PSQI) and its reliability coefficient was 0.88. On the same day, self-reporting questionnaires concerning stress, obtained from the Department of Mental Health, Ministry of Public Health, Thailand, were filled in by the workers. All questionnaires were completed 30 minutes before the morning shift began.

Sampling and collecting

A saliva collector (Sartsert, Germany) was used to collect the saliva samples. Twenty-four hour saliva collection was separated into two sections; during working hours at the factory and during off-work hours at the workers' houses. The morning shift was from 07:00 to 15:00 and saliva was collected at 07:00, 10:00 and 13:00 at the work place, and at home at 16:00, 19:00, 22:00, 01:00 and 04:00. Saliva collection for the night shift workers was performed at 01:00, 04:00, and 07:00 at the work place and at 10:00, 13:00, 16:00, 19:00 and 22:00 at home. All samples were frozen at -20°C. WBGT indexes and light intensity were measured at 07:00 and 13:00 during the morning shift and at 23:00, 01:00 and 04:00 during the night shift.

Biochemical analysis

Saliva melatonin was measured by ELISA (Microplate reader model ELNX 96) using a direct saliva melatonin test kit (BÜHLMANN Laboratories AG, Switzerland). The test sensitivity was 1.5 ± 0.2 pg/ml. For all analyses, samples obtained for each shift from each

subject were measured using the same assay.

Data and statistical analyses

Saliva samples were taken every three hours and used to determine the subjects' melatonin profiles. Saliva melatonin data from the morning and night shifts were compared and evaluated by the Wilcoxon signed ranks test. Differences in saliva melatonin levels in the younger and older subjects were evaluated by the Mann-Whitney *U* test, as was the difference in saliva melatonin at each stress level. A *p*-value <0.05 was considered to be significant.

RESULTS

Subject characteristics

Subject characteristics are shown in Table 1. Most subjects were married. None smoked, but four sometimes consumed alcohol. Eight were taking oral contraceptives. Two of the older subjects had gastritis within the year prior to the study. Two different stress levels were found, with six subjects having normal stress and four having mild stress.

Working conditions

The workplace was an open area with two floors and a high ceiling. Natural ventilation and mechanical general ventilation were used to improve air quality. The WBGT heat stress index was 27.88-28.23°C WBGT during the morning shift and 26.85-27.38°C WBGT during the night shift. All subjects were classified as having light workloads. The permissible heat exposure threshold limit value for light workload and continuous work is 30°C. Therefore heat stress probably did not occur. During the morning shift, there was both natural and artificial light, but during the night shift there was only artificial light. The light intensity was 1,333.4-1,617.6 lux during the morning shift and 1,611-1,727 lux during the night shift. There was no significant difference be-

Table 1
General characteristics of subjects.

Characteristics	Younger group n = 5	Older group n = 5
Age (years)		
mean ± SD	21.4 ± 0.55	38.4 ± 1.82
Working experience (years)		
mean ± SD	2.22 ± 1.57	15.6 ± 5.13
Marital status		
Married	5	4
Divorced	0	1
Smoking		
No	5	5
Drinking		
Yes	0	4
No	5	1
Medication (Oral contraception)		
Yes	5	3
No	0	2
Health status in the past year		
Normal	5	2
Allergy	0	1
Gastritis	0	2
Stress level		
Normal	2	4
Mild	3	1

tween the morning and night shifts in terms of light intensity. The workplace had adequate light intensity for performing fine detail work without eye strain.

Saliva melatonin

Figs 1 and 2 show the data from saliva collected every three hours from subjects during the morning and night shifts. During the morning shift the melatonin level of the older subjects was lowest at 10:00 (2.32 pg/ml). The onset of increased melatonin production occurred at 19:00 (3.19 pg/ml), with a peak level at 01:00 (12.8 pg/ml). In the younger subjects the onset of melatonin production was also at 19:00 (6.43 pg/ml) but the highest levels occurred later, at 4:00 (32.37 pg/ml). The low-

est value was 2.37 pg/ml at 13:00. The onset time of saliva melatonin profiles in younger and older subjects during the night shift began gradually at 19:00 (3.89 and 1.11 pg/ml in younger and older subjects, respectively) and reached a maximum level at 22:00 (13.41, 17.4 pg/ml in the younger and older subjects, respectively).

Melatonin levels in the older group were compared between the two shifts (morning and night shift), with a significant ($p < 0.05$) difference noted at 07:00. In younger subjects melatonin concentrations differed significantly between the morning and night shift sampling periods at 19:00.

There was no significant difference in melatonin levels between younger and older groups in the morning shift at any time measurements were made. For the night shift, there was a significant difference between the younger and older subjects in melatonin levels at 19:00. The onset and offset times were at approximately 19:00 and 07:00, respectively.

Melatonin and stress

The comparison of saliva melatonin between the normal and mild stress groups during the morning shift is shown in Fig 3. There was no significant difference between melatonin concentrations in these groups.

DISCUSSION

This study was designed to determine the profiles of melatonin and stress in shift workers by assessing melatonin levels in saliva and stress levels using a stress questionnaire.

The general health status of shift workers was normal. The sleep/wake cycle of shift workers was more than six hours if they did not work a night shift. However, they slept only four hours (range 2-8) after completing the shift.

This field study was conducted in a glass

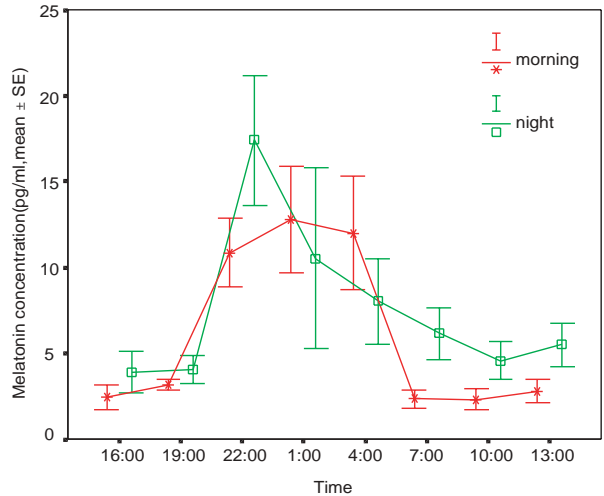


Fig 1—Comparison of saliva melatonin levels between morning and night shifts in older subjects. The data are expressed as the mean ± SE pg/ml

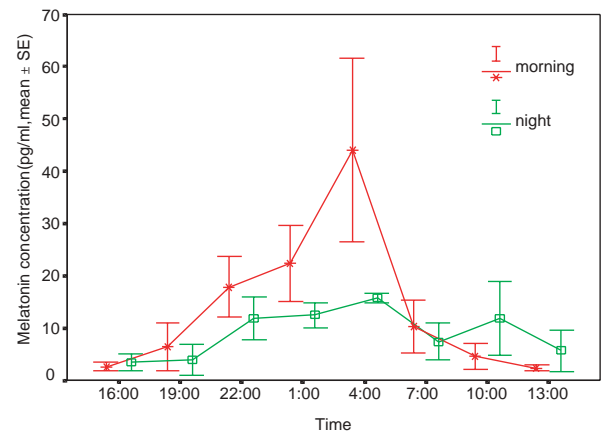


Fig 2—Comparison of melatonin levels between morning and night shifts in younger subjects. The data are expressed as the mean ± SE pg/ml.

factory. The results differed from previous laboratory studies (Härmä *et al*, 1994; Voultsios *et al*, 1997; Gibbs *et al*, 2002). The advantage of laboratory testing is that it is able to control confounding factors and random error. To control flow of saliva, cotton swabs without citric acid were used to collect saliva, therefore swabs did not influence melatonin concentra-

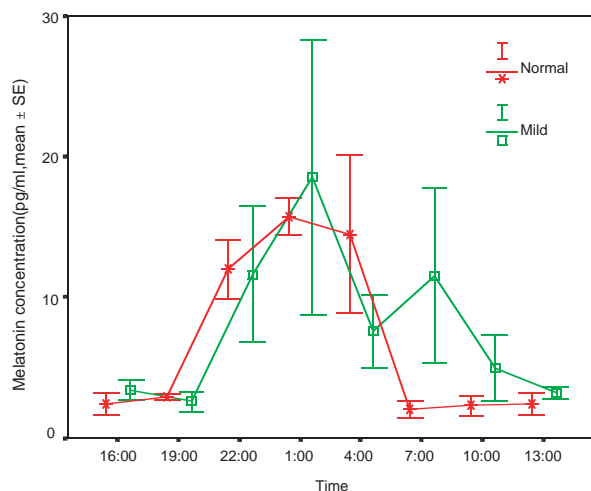


Fig 3—Comparison of melatonin levels in the normal and mild stress groups. Data are expressed as the mean \pm SE pg/ml.

tions. Food, however, was a confounding factor due to increases in melatonin levels caused by food intake (Leibowitz and Alexander, 1998). Morning shift workers had a set break time for lunch, but the night shift workers did not, instead breaking for meals anytime they wished during their working hours. For this reason, melatonin synthesized from the worker's food intake has a greater influence than other factors. The food intake protocol was introduced to control the types of food consumed by subjects.

Light intensity was the primary factor suppressing melatonin secretion (McIntyre *et al*, 1989; Hashimoto *et al*, 1996; Aoki *et al*, 1998). During the night shift, light intensity was the same as during the daytime. A suppressing process may occur in the older group more than in the younger group. This is because melatonin levels decreased after 01:00 in the older group, but in the younger group melatonin concentrations were steady from 22:00 to 04:00. Another explanation may come from several studies that found either advance or delay in the phase of melatonin profiles of shift

workers (Waterhouse *et al*, 1989; Härmä *et al*, 1994; Roman, 1997; Barnes *et al*, 1998) due to work schedule. During the morning shift there were no significant differences between melatonin levels in the younger and older groups. It is possible that the range in age of the subjects was too close to show any differences by age. Another study (Härmä *et al*, 1994) using subjects with a greater age range found a significant difference in melatonin concentrations among age groups. Melatonin concentrations declined late at night, especially in patients with depression or alcoholism, and their melatonin levels appeared to be lower than in the normal population (Rohr and Herold, 2002). However, in this study the melatonin concentrations of shift workers with normal and mild stress were not significantly different throughout the 24-hour day. This may be due to the degree of stress, which in this study was mild.

Finally, further studies of melatonin in shift workers need to control food intake. To confirm the relationship between melatonin and stress, subjects with depression or severe stress should be included. Length of shift rotation and melatonin profile should be observed over a long period to better understand how these factors influence melatonin secretion.

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