Analysis of salivary cortisol levels to determine the association between depression level and differences in circadian rhythms of shift-working nurses

Michie Baba[1], Mitsuru Ohkura[2], Kayoko Koga[1], Kyoko Nishiuchi[3], Lourdes R. Herrera C.[2], Ryoichi Matsuse[4] and Toshitaka Inoue[5]

[1]School of Nursing, Faculty of Medicine, Fukuoka University, Japan, [2]Japanese Red Cross Kyushu International College of Nursing, Japan, [3]Faculty of Nursing, Baika Women's University, Japan, [4]Kyoto Ikagaku Co. Ltd., Japan and [5]Inoue Hospital, Japan

Abstract: Analysis of salivary cortisol levels to determine the association between depression level and differences in circadian rhythms of shift-working nurses: Michie Baba, *et al*. School of Nursing, Faculty of Medicine, Fukuoka University—Objective: The aim of this study was to clarify whether there are differences in the circadian rhythms of shift-working nurses by assessing depression, fatigue and salivary cortisol levels. **Methods:** Forty nurses working in a two-shift system at "Hospital A", Fukuoka City, Japan, used a self-rated depression scale (SDS) to assess their depression levels. Fatigue levels were measured with the visual analogue scale for fatigue (VAS-F); saliva was collected before and during shifts for three days. Results were analyzed with analysis of variance (ANOVA). **Results:** Thirty-six valid records were obtained, and subjects were classified according to SDS scores into a normal group (NG), moderate group (MG) and severe group (SG). There were no significant differences in the day shift salivary cortisol values of the three groups. However, the night shift salivary cortisol value for the SG was 0.132 μg/d*l* at 16:00, before starting the shift, and decreased to 0.036 μg/d*l* at 20:00. It increased slightly up to 0.057 μg/d*l* by 24:00 and formed a peak between 5:00 and 7:00, with the levels being 0.322 μg/d*l* and 0.305 μg/d*l* respectively. Meanwhile, the NG cortisol value was 0.154 μg/d*l* before the shift, decreased to 0.034 μg/d*l* by 20:00, slightly increased up to 0.093 μg/d*l* by 5:00 and presented its peak value, 0.253 μg/d*l*, at 7:00 next morning. **Conclusions:** SG nurses presented significantly increased salivary cortisol levels early in the morning during night shifts, showing a phase deviation in the circadian rhythm. Because subjective fatigue levels did not differ with time, SG nurses should understand and deal with physical changes in the early morning. This approach may reduce medical accidents and malpractice in the early morning.

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Humans have an endogenous circadian clock (internal body clock with a 24-hour cycle) called a circadian rhythm. The circadian rhythm is a regular cycle of 23–25 hours, and this rhythm is maintained even when affected by external stimuli[1–5]. The circadian rhythm is essential for the regulation of such things as neural activities, immune functions, and sleep; importantly, it is connected with autonomic nervous system functions that include regulation of blood pressure, body temperature, metabolism; and endocrine secretion[2,5–8]. Today, as society has diversified, the numbers of individuals working night shifts and shift workers have increased. Since two-shift workers have to work both a day shift and night shift in turn, the synchronization of their biological rhythms is difficult. Previous studies reported that shift work causes sleep disorder, fatigue, anxiety, nervous temperament, and depression[6–20].

Nurses at hospitals with inpatient facilities have to work in shifts to provide adequate 24-hour care to patients. Some studies reported that, in addition to being easily tired, night-shift nurses working in a two-shift system suffer from anxiety in trying to avoid mistakes and are susceptible to depression due to this stress[9–12]. Night-shift nurses who work against the circadian rhythm are prone to fatigue and decreased work performance, resulting in occurrences of medi-

cal accidents and errors at night[13–20]. Moreover, some studies have reported that errors and medical incidents during work by nurses with depression tend to happen particularly in the early morning[21,22].

Although it has been noted in a large number of studies that shift-working nurses are subject to depression, and that medical errors and incidents are caused by them more frequently in the early morning when they are on a night shift, the factors associated with these tendencies have not been objectively examined.

Considering this background, this study examined differences in the circadian rhythm among different degrees of depression in shift-working nurses.

The Self-Rating Depression Scale was used to measure the depression level. Furthermore, we measured the salivary cortisol level as an objective indicator of the circadian rhythm[23–32]. We also investigated the subjective physical fatigue and subjective mental fatigue in the nurses. If there is a difference in objective circadian rhythm depending on depression levels, then we could examine coping methods in accordance with the situation, which could help to avoid errors and medical incidents that are common in nurses working on a two-shift system.

Subjects and Methods

Subjects

The subjects were 40 nurses with ≥ 3 years of work experience who were working in a two-shift system at a chronic ward in "Hospital A", which is located in the southwestern part of Fukuoka City, Japan. The two-shift system consists of an approximately 16-hour night shift (from 16:30 to 8:30 the following day, with a 2-hour nap/break) 1 to 3 times per month and an 8-hour day shift (from 8:00 to 17:00). For an average of approximately 37 patients, 10−13 nurses work on each day shift, and 3 nurses work on each night shift. Although emergency admission of patients to this ward is rare since it is a chronic ward, a sudden change in patients occurs at midnight 1−2 times per month. Nurses do not work consecutive night shifts. They work 1−3 night shifts per month.

We limited the subjects to those who had ≥3 years of nursing experience based on previous studies that indicated that shift-work nurses achieved adaptation to the night-shift process in 3 years, during which body temperature increases are suppressed and metabolism is depressed. Thus, compared with the time when the nurses were new to the job, their subjective lack of sleep was improved[13].

Survey items and method

This survey was conducted in March and April 2011. The study purpose and method were explained to the potential subjects candidates after their day shift in early March. Those who consented to the study were administered a questionnaire regarding 1) basic information and 2) depression level. Thereafter, their salivary cortisol and subjective fatigue levels (subjective physical fatigue and subjective mental fatigue) were measured at each designated time for 3 consecutive days on which the nurses worked a day shift on day 1, started a night shift on day 2 and then completed the night shift in the morning on day 3. Saliva collection was performed on the day after a day shift.

Ten measurements were taken (day 1, 8:00 before day shift, 12:00, and 17:00; day 2, 16:00 before night shift, 18:00, 20:00, and 24:00; day 3, 5:00, 7:00; and 9:00 after night shift).

Basic information

Basic data collected in the questionnaire included gender, age, duration of work experience as a nurse, duration of work experience at "Hospital A", and subjective health status. Questions on factors that might influence salivary cortisol level were also included, namely, current use of oral medications and their names, smoking/nonsmoking habits, and whether they took night-shift naps.

Level of depression

The Self-Rating Depression Scale (SDS) developed by Zung was used to measure the depression level. The SDS is widely used by the National Institute for Occupational Safety and Health (NIOSH) in the US to measure depression. The advantages of this scale include ease of use in self-rating and its applicability to a wide range of people, from healthy individuals to patients. The SDS assesses the presence or absence of symptoms associated with depression in the preceding week by using 20 items, each of which is scored on a scale from 1 to 4 ("rarely", "sometimes", "usually", and "mostly"). This study used a Japanese version of the SDS translated by Fukuda *et al.*[23]. The reliability and credibility of this version have been examined and reported.

Fukuda *et al.* assessed the levels of depression by score: 39 points or less indicates "little or no depression", 40−49 points indicates "mildly depressed", and 50 points or greater indicates "moderately depressed." In the present study, the depression levels were classified by SDS score into three groups: "normal Group" (NG) (39 points or less), "moderate Group" (MG) (40−49 points), and "severe Group" (SG) (50 points or more).

Salivary cortisol

Salivary cortisol levels were measured as physiological indexes of circadian rhythms and stress responses. Cortisol is a hormone secreted by the adrenal gland.

Because it has various physiological influences on the immune, blood circulatory, and central nervous systems, cortisol is an important hormone to consider when determining physical and mental health[22,23]. Since salivary cortisol is highly correlated with blood cortisol level, and saliva can be sampled less invasively than blood, it is often used in studies on circadian rhythm and work-related stress[24–32]. In addition, salivary cortisol is easy to use, as the sampling of saliva requires no particular time or place, and multiple samplings are possible[30–32]. Therefore, we examined the circadian rhythm by measuring the salivary cortisol level.

Prior to measuring the salivary cortisol level, a pretest was conducted to determine the physiological index items in saliva that serve as stress indexes, and to identify the optimal time to sample saliva during working hours[31]. We found that salivary cortisol was the most effective stress index. Salivette kits were used to collect saliva from the subjects. The saliva sampling procedure was explained to the 40 nurses, and they were given the required number of Salivette kits in advance. Saliva was collected at each predetermined time by using a cotton pledget from the kit. The pledget was placed under the tongue for 2 minutes, until it was soaked in saliva, and then it was placed in the Salivette tube (Sarstedt, Nümbrecht, Germany). Immediately after saliva collection, used Salivette tubes were cryopreserved in a freezer placed in each ward. Subsequently, they were collected and sent frozen, with dry ice, together to Kyoto Laboratory for analysis. The laboratory further froze the received tubes at −80°C, and centrifuged them at 1,500 g for 10 minutes at 20°C. The salivary cortisol level was then assessed using a High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics LLC, State College, PA, USA) and Multiskan MS-UV 96-well plate reader, (Labsystems, Helsinki, Finland) according to their instructions. The subjects were instructed to refrain from eating, smoking, brushing their teeth, and gargling for 30 minutes before each saliva sampling. The subjects were allowed to provide samples up to 30 minutes before or after the designated time.

Subjective fatigue

Physical and mental subjective fatigue levels were recorded separately on a visual analogue scale (VAS) of 0 to 10. On this linear scale, the far left end denoted "no fatigue at all" with a rating of 0, and the right end indicated "extremely fatigued" with a rating of 10. The subjects were asked to circle the position that best described their current subjective fatigue level.

Analysis method

The salivary cortisol levels, and subjective physical fatigue and subjective mental fatigue scores were compared for the three groups: NG, MG; and SG. First, two-way repeated measures analysis of variance was performed to examine correlations among the 3 groups. Thereafter, a simple main effects analysis was performed by one-way analysis of variance (ANOVA). After one-way ANOVA was conducted among the three groups, Dunnett's test was performed on the MG and SG with reference to the NG. PASW Statistics ver.18.0 was used for statistical analysis, with the significance level set at $p<0.05$.

Ethical considerations

After permission was obtained from the director of "Hospital A" and the director of nursing, the purpose and methods of the study were explained to the subjects orally and in writing, and their written consent was obtained. This study was approved by the Ethics Committee of Fukuoka University prior to implementation.

Results

Responses from 36 subjects who completed all the samplings were considered valid. Of these, one subject taking oral cold medicine that may affect salivary cortisol levels, according to the basic information questionnaire, excluded.

Overview of the subjects (Table 1)

The average SDS score was 43.4; 10 subjects (27.7%) were in the NG, 19 (52.8%) were in the MG, and 7 (19.4%) were in the SG.

Of the 36 subjects, 4 were male and 32 were female (mean age: 36.9 years). Their average duration of work experience as a nurse was 13.8 years, and their average duration of employment at "Hospital A" was 6.9 years. Regarding subjective health status, 34 (94.4%) subjects were healthy. There were no significant differences among the three groups in terms of age, years of work experience as a nurse, or subjective health status. In addition, there were no differences with respect to smoking or night-shift naps.

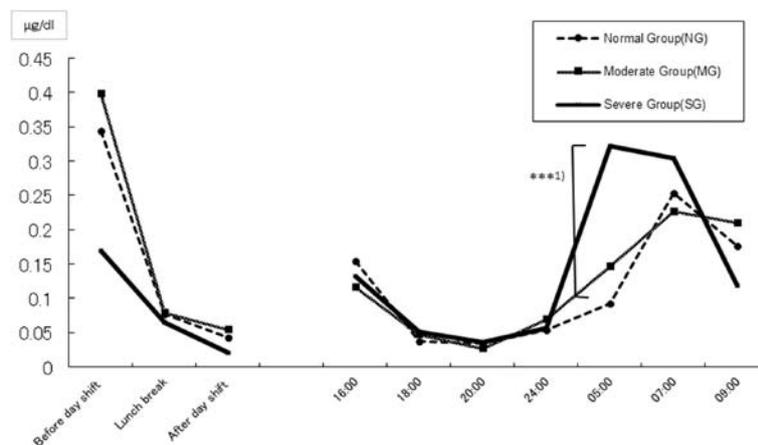*Changes in salivary cortisol levels by depression level*

Figure 1 shows the changes in salivary cortisol levels according to the depression level. The salivary cortisol levels during the day shift were lowest in the SG, followed in ascending order by those in the NG and MG.

The salivary cortisol levels in the SG during the day shift were highest, at 0.170 µg/dl, before starting the day shift, declining to 0.065 µg/dl by the lunch break and 0.021 µg/dl after completing the day

Table 1. Overview of subjects according to the depression level

			Depression level				
		Total n=36	Normal Group (NG) n=10	Moderate Group (MG) n=19		Severe Group (SG) n=7	$p^{1)}$
Gender	Male	4	4 (40.0%)	0 (0.0%)		0 (0.0%)	**
	Female	32	6 (60.0%)	19 (100%)		7 (100%)	
Age (years)	23–29	10	2 (20.0%)	6 (31.6%)		2 (28.6%)	ns
	30–39	15	5 (50.0%)	7 (36.8%)		3 (42.9%)	
	40–57	11	3 (30.0%)	6 (31.6%)		2 (28.6%)	
Nursing experience (years)	Total years of experience	13.8 ± 8.70	13.5 ± 8.48	14.2 ± 7.52	$ns^{2)}$	13.5 ± 12.79	$ns^{3)}$
	Experience at 'Hospital A'	6.9 ± 8.03	5.6 ± 7.06	6.4 ± 6.42	$ns^{2)}$	10.5 ± 12.62	$ns^{3)}$
Subjective health status	Healthy	32	10 (100.0%)	18 (94.7%)		4 (57.1%)	ns
	Moderately healthy	3	0 (0.0%)	1 (5.3%)		2 (28.6%)	
	Not healthy	1	0 (0.0%)	0 (0.0%)		1 (14.3%)	
Smoking	Yes	10	5 (50.0%)	2 (10.5%)		3 (42.9%)	ns
	No	26	5 (50.0%)	17 (89.5%)		4 (57.1%)	
Night-shift nap	Yes	20	4 (40.0%)	11 (57.9%)		5 (71.4%)	ns
	No	16	6 (60.0%)	8 (42.1%)		2 (28.6%)	

χ^2 was performed. **: p<0.01, ns: Not significant. After ANOVA was performed among the 3 groups, Dunnett's test was conducted on the Mild and Moderate Groups. **: $p<0.01$, ns: Not significant. After ANOVA was performed among the 3 groups, Dunnett's test was conducted on the Mild and Severe Groups. **: $p<0.01$, ns: Not significant.

Fig. 1. Changes in saliva cortisol level according to depression level.

shift. The NG also showed the salivary cortisol levels decreasing from 0.344 μg/d*l* before the day shift; to 0.077 μg/d*l* by the lunch break and then 0.043 μg/d*l* after the day shift. The same tendency was observed in the MG.

Regarding the salivary cortisol levels during the night shift, the SG showed a level of 0.132 μg/d*l* at 16:00 before the start of the shift, and the level decreased to 0.036 μg/d*l* by 20:00. The level slowly increased to 0.057 μg/d*l* by 24:00. Subsequently, it continued to increase the next morning, peaked for

2 hours from 5:00 to 7:00, at 0.322 and 0.305 μg/d*l*, respectively, and again decreased to 0.176 μg/d*l* by 9:00. The NG showed a level of 0.154 μg/d*l* at 16:00 before the start of the shift, and the level decreased to 0.034 μg/d*l* by 20:00. However, the levels gradually increased to 0.093 μg/d*l* by 5:00 the next morning, peaked at 0.253 μg/d*l* at 7:00, and again decreased to 0.118 μg/d*l* by 9:00. The same tendency as in the NG was observed in the MG.

Up to this point, the changes in the SG during the day shift were similar to those in the NG and
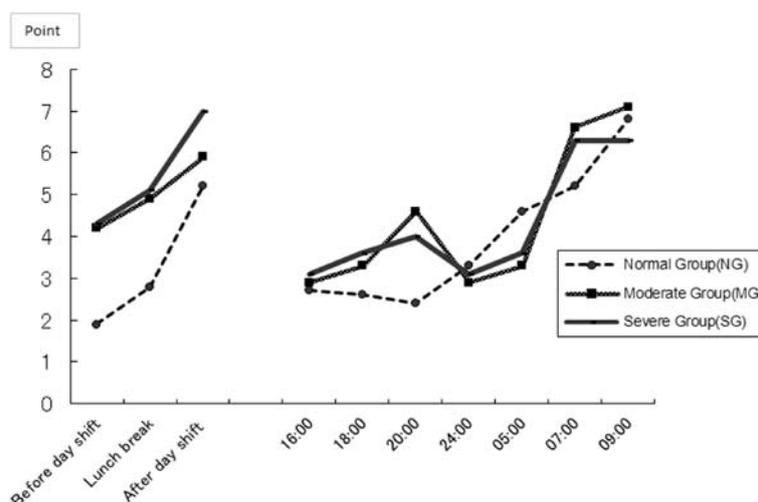
Fig. 2. Subjective physical fatigue according to depression level.

MG. However, as correlations were observed in the two-way ANOVA during the night shift (from 24:00 to 9:00) ($p<0.05$), one-way analysis of variance was performed, and the salivary cortisol level was found to be markedly higher in the SG than in the NG and MG at 5:00 in the morning.

Subjective physical fatigue and subjective mental fatigue by depression level

Figure 2 shows subjective physical fatigue according to the depression level. The subjective physical fatigue score during the day shift was as high as 4.3 in the SG before work, linearly increased with time, and reached 7.0 on completion of the shift. A similar tendency was observed in the MG. The NG's subjective physical fatigue was 1.9 before starting the day shift, and it linearly increased over time, reaching as high as 5.2 by the end of the day shift.

The subjective physical fatigue scores during the night shift were similar among the 3 groups, approximately 3, at 16:00 before work. Subsequently, in the SG, after showing 4.0 at 20:00, it decreased to 3.1 by 24:00, and continuously increased until 9:00 on the next day, with the scores being 3.6 at 5:00 and 6.3 at 7:00. A similar tendency was observed in the MG. In contrast, in the NG, the score remained unchanged until 24:00 after the start of the shift; but continuously increased to 4.6 at 5:00, 5.2 at 7:00; and 6.8 at 9:00 on the next day. There were no significant differences in subjective physical fatigue at any time point during the day or night shift between the MG and NG compared with the SG.

Figure 3 shows subjective mental fatigue according to the depression level. The NG scored the lowest in day-shift subjective mental fatigue, followed in ascending order by the MG and SG. The SG's

subjective mental fatigue was 4.9 before starting the day shift, increased linearly over time, and reached as high as 6.7 by the end of the shift. The MG's scores at the start and end of the day shift were 3.9 and 5.9, and the NG's scores at the start and end of the day shift were 1.3 and 4.5, respectively.

The SG's score was 3.4 at 16:00 before starting the shift, and remained at the same level until 24:00. Thereafter, it increased to 4.7 by 5:00 the next morning, and continued to increase to 5.4 by 9:00. The NG showed a gradual increase in score from 2.0 at 16:00 to 4.5 by 7:00 the next morning; and then to 5.4 by 9:00. There were no significant differences in subjective mental fatigue at any time point zone during the day or night shift between the MG and NG compared with the SG.

Discussion

The present study aimed to determine whether there were differences in circadian rhythm depending on depression level in nurses who work in a two-shift system. The mean age of the subjects was 36.9 years. Their average work experience as a nurse was 13.8 years, and there were fewer nurses in their 20s and 50s than in other age groups. On the basis of the basic information provided, the subjects were considered to be accustomed to nursing work to some extent. The subjects' average depression level was 43.4 SDS points, which was higher than the 35 points of the control group of the general population in the study of Fukuda *et al*[23]. The previous SDS surveys for nurses found 40 or more points in 70% of the respondents, and nurses were considered to have lower mental well-being than the general population[33,34]. The present SDS survey also showed 40 or more points in 69.9% of the subjects, which coincides
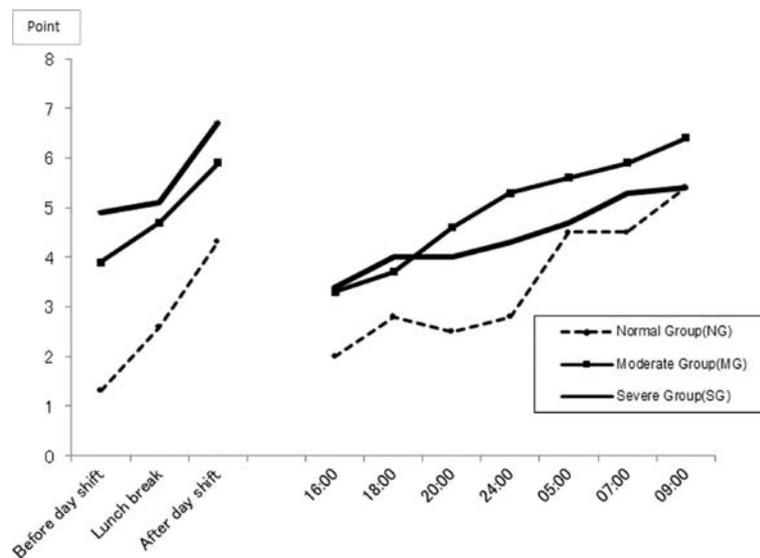
Fig. 3. Subjective mental fatigue according to depression level.

with previous study findings. Thus, the subjects were considered to be of the general nurse population.

We examined whether the circadian rhythm in the nurses differed depending on their depression levels by measuring salivary cortisol levels. The human circadian rhythm has a regular cycle of 23 to 25 hours, and this rhythm is maintained even under external influence. According to Nakamura, cortisol secreted from the latter half of the sleeping phase to the time of waking up time promotes gluconeogenesis during the period without energy supply (from the sleeping phase to time of waking up) to allow the body to be in an active state[1]. It is believed that the usual sleep-wake rhythm is maintained even during night shifts. Miyauchi measured blood cortisol levels from the day shift to the night shift in nurses and found that the levels peaked at 8:00, decreased in the evening; and further decreased at night; a rhythm was observed, with the levels elevated suddenly from 6:00 to 8:00 next morning[37].

Our results are similar to the results of these studies; the salivary cortisol level was higher just after starting the day shift and decreased toward evening. The same rhythm form was observed in the night shift: it continually decreased until 24:00, and increased suddenly the next morning. In regard to a nurse's duties, only a limited number of night-shift nurses handled preparations for examinations, vital sign checking, and preparations for breakfast; beginning at 6:00, which resulted in peaks of busyness and stress. Izawa reported that cortisol levels were increased by physical stress and acute mental stress, and that a literature review indicated that the levels were affected by chronic mental stress[24]. Izawa also

reported that salivary cortisol increased in the state of greatest strain (high work demand and low control[1], and Kato reported that salivary cortisol increased before work because of tension and stress[30,31].

However, there was a difference in the rhythm in the early morning depending on the depression level, and high cortisol levels were found from 7:00 to 9:00 in the NG and MG, which is similar to the results of Miyauchi[37]. In contrast, in the SG, the levels were elevated as early as 5:00, two hours earlier, and the cortisol-releasing status was prolonged for as long as 2 hours. However, in the present study, a rapid increase in the cortisol level was observed earlier, at 5:00, in the SG than in the NG and MG, and was maintained until 7:00, indicating an impaired circadian rhythm that may have increased mental tension and stress earlier in this group than in the other 2 before work.

As night-shift nurses have to start their duties earlier than general-shift nurses, they are in a state similar to jet lag, which results in fatigue, sleeplessness, and impaired concentration, thereby increasing the likelihood of mistakes[13–20].

It is said that there is a relationship between circadian rhythm disorders and depression[25]. Furthermore, nurses showing higher depression levels have been reported to cause medical errors or incidents more frequently in the early morning[26]. On the other hand, in the comparison of subjective fatigue at 5:00, no differences were observed among the 3 groups. Murayama reported that many cases of clinical depression may have been overlooked due to the absence of subjective symptoms[38].

The results of the present study suggest that night-

shift nurses may be implementing their duties in the early morning without appropriately recognizing the presence of increased fatigue or stress due to an impaired circadian rhythm.

Nurses are expected to respond to social expectations as interpersonal service specialists and to possess a sense of duty as nursing attendants[35], as well as to care for their own health and restrict their effects on patients[36]. This may prevent them from self-reporting the presence of fatigue. As future perspectives, it may be important for nurses to confirm the presence/absence of depression through daily self-assessment. In the case of high depression levels, they should manage themselves by alleviating tension using techniques, such as relaxing, even if subjective symptoms are absent, and receiving training to express affirmatively their sense of physical and mental fatigue to others, rather than simply suppressing it. Although they can individually make these efforts, it may be necessary to establish systems for the early identification of nurses with high depression levels and create environments enabling nurses at work to relax in the early morning and express their sense of fatigue[39]. These approaches may contribute to the prevention of medical errors caused by shift-working nurses.

Limitations

In measuring salivary cortisol level, concern is often raised that there may be sampling bias due to insufficient management of saliva sampling and sampling times. For this study, we believe that compliance was observed and that sampling bias was minimized. This was possible due to the main survey being conducted after the pretest, and because the researchers could thoroughly explain and ensure that the sampling procedures and sample measuring and storing methods were correct, since the study covered only one hospital with two wards.

This study was conducted at one medical institution with a limited number of samples. Therefore, further examination is needed to conclude whether the study findings can be considered as representative of the characteristics of shift-working nurses. Additionally, further studies investigating regular sleeping hours and sleeping habits, which were not included in this study, are necessary. A future issue is confirmation of the findings of this study while conducting intervention studies.

In the SG working in a two-shift system, a significant increase in salivary cortisol level was found in the early morning during the night shift, which showed a phase shift of the circadian rhythm in early the morning. However, considering that there was no difference in the subjective fatigue levels according to time, the nurses in this group may not understand

their own physical changes in the early morning, so further studies exploring this aspect should be undertaken.

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