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Differences between Hair Cortisol Concentrations in Day Shift Workers and Rotating Night Shift Workers in Japan

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Abstract

Purpose: The study aimed to determine the differences between stress levels in day shift workers and night shift workers by measurements of hair cortisol concentration (HCC) and by using self-administered questionnaires.

Methods: HCC was measured by using liquid chromatography-mass spectrometry. The subjective stress level was evaluated by a brief job stress questionnaire, stress response scale-18 (SRS-18), and visual analog scale (VAS).

Results: Mean (± standard deviation: SD) HCC in the 16 subjects was 17.28 ± 7.39 pg/mg. There was no significant difference between HCCs in day shift workers (17.98 ± 3.03 pg/mg) and rotating night shift workers (16.37 ± 1.86 pg/mg). There were also no significant differences in SRS-18 scores, job-related stress scale scores, and VAS scores between day shift workers and rotating night shift workers. There was a significant difference in HCC between the group in which the stress condition was weak or normal and the group in which the stress condition was slightly strong or definitely strong according to the SRS-18 level ($p = 0.030$).

Conclusions: Day shift workers and rotating night shift workers have similar HCCs and similar degrees of job-related stress. In rotating shift workers who feel strong stress, acquirement of resilience due to stress coping for medium- to long-term stress may be involved in low HCC.

Keywords: Hair cortisol concentration, job-related stress, shift worker, day worker

1. Introduction

Many workers are involved in rotating shift work that operates around the clock. Rotating shift workers have a physical overload due to the repetition of day and night shifts, and the hours that rotating shift workers spend awake are different from those of their families and society in general. It has been reported that rotating shift workers have large physical, psychological and social stresses. It has also been reported that rotating shift workers are likely to have chronic general fatigue, sleep disturbance an increase in stress-related diastolic blood pressure and the occurrence of gastroesophageal reflux disease (Kida & Takemura, 2022; Madeira, Fernandes, Paiva, Moreira, & Caldeira, 2021; Najafimehr et al., 2018).

Measurement of cortisol level has been used for evaluation of stress. Cortisol levels in serum and saliva reflect short-term stress states. On the other hand, hair cortisol concentration (HCC) has recently been used as a medium- to long-term stress marker in many studies. Stalder et al. showed in a meta-analysis that HCCs were positively associated with stress-related anthropometric factors such as body mass index and waist-to-hip ratio and with systolic blood pressure (Stalder et al., 2017). Meyer et al. used HCC measurements to investigate the effects of significant life stressors on chronic hypothalamic-pituitary-adrenal (HPA) activity and reported that the size of HCC provides a new approach for assessing long-term HPA activity over weeks to months (Meyer & Novak, 2012).

There have been several studies on the relationships between high HCC and work-related stress (Schaafsma et al., 2020) and high HCC and work-related values (Steinisch et al., 2014), and HCC is shown to be involved in the relationship between job burnout and insomnia (Wang, Dai, & Li, 2019). For shift workers, it is important to evaluate medium- to long-term stress states due to the continuation of work. Results of studies on HCCs in day shift workers and rotating shift workers have been controversial. It has been reported that HCC in shift workers

was significantly higher than that in fixed day shift workers (Zhang et al., 2020) and that shift workers had a significantly higher HCC than that in day workers at a younger age (Manenschijn et al. 2011). However, Janssens et al. showed in an analysis adjusted for an age that HCC in shift workers was significantly lower than those in day workers (Janssens et al., 2017). To our knowledge, there has been no study on HCC in male rotating shift workers in Japan. The study aimed to determine the differences between stress levels in day shift workers and night shift workers by measurements of HCCs and by using self-administered questionnaires.

2. Materials and Methods

The survey was conducted in January 2022. We recruited day shift workers and rotating night shift workers among men working in the manufacturing industry. We excluded workers with dyed hair, workers who were taking steroid hormones and workers in whom the length of hair in the back of the head was less than 3 cm. We informed the aim of the study to an occupational health nurse in the company and asked a factory manager to cooperate and obtain approval for the study. After an explaining the study, we obtained written informed consent from 16 workers.

We used HCC as a biological biomarker and used questionnaires including three psychological stress scales. For the measurement of HCC, we obtained 10-15 hairs and measured HCC by using liquid chromatography-mass spectrometry (LC-MS/MS) at Aska Medical Co. Ltd (Hu & Lodewijks, 2020; Soares, Antunes, & Linden, 2021; van Zundert et al, 2022). The hair samples after washing with 2-propanol were crushed into powder, and an internal standard was put to them. An aliquot of 0.5 mL trifluoroacetic acid and 50% acetonitrile solution (0.1 M) was put to the pulverized hair and incubated at 40°C for 1 hour. Then, 4 mL of methyl tert-butyl ether was added and the mixture was shaken. The organic layer was then separated and dried with a centrifugal evaporator. An aliquot of 0.5 mL methanol and 1 mL water were consecutively added to the residue before purifying it with an Oasis MAX cartridge, a mixed-mode type solid-phase extraction column. The steroid fraction was eluted and dried with a centrifugal evaporator, and the fraction derivatized was purified on a HyperSep SI cartridge. Subsequently, the eluent was evaporated and the residue was dissolved in 100 µL of 40% acetonitrile solution. Twenty µL of sample solution was sequentially subjected to quantitation in LC-MS/MS. The range of measurement was 2-1000 pg/ml. The intra- and inter-assay coefficients (CVs) were 6.5% and 10.6%, respectively.

Stress response scale-18 (SRS-18), visual analog scale (VAS), and job-related stress scale as psychological stress scales were used. SRS-18, which is a scale for the measurement of the psychological stress response, was developed by Suzuki et al. SRS-18 consists of 3 categories and 18 items. Short-term stress can be assessed by using SRS-18. Stress was evaluated by a 4-grade scale including low (≦ 7 points), normal (8–19 points), slightly high (20–31 points), and high (≧ 32 points). SRS-18 has three subscale categories including depression-anxiety, irritability-anger, and helplessness. There have been several studies on the evaluation of stress responses by using the SRS-18 in university students in Japan (Kusumoto, Higo, & Ohno, 2022; Honda, Yoshikawa, Yamashina, Yamato, Terada, & Goto, 2020). We used VAS for the evaluation of total stress strength in day shift work and night shift work. Job-related stress factors were assessed by the Brief Job Stress Questionnaire, which was provided by the Ministry of Labour in Japan (The Ministry of Labour). There were 17 questions in 9 categories. Items regarding quantitative overload, qualitative overload, job control, and interpersonal relationships were evaluated on a 5-point scale and items regarding physical overload, workplace environment, skill discretion, job fitness, and job satisfaction with work were evaluated on a 4-point scale.

2.1 Statistical Analysis

Analysis was performed by using SPSS (IBM, Version 25). HCCs and SRS-18 scores in day shift workers and rotating night shift workers were compared by using the t-test after confirmation of normal distributions by the Shapiro-Wilk test. Levels of job-related stress in day shift workers and rotating night shift workers were compared by using the Kruskal-Wallis test. VAS scores in day shift workers and rotating night shift workers were compared using the Mann-Whitney U test. Correlations of HCC with age, job-related stress scale scores, and SRS-18 scores were evaluated by Spearman's rank correlation coefficients.

2.2 Ethical Consideration

This study was approved by the Research Ethics Committee of the University (approval number: 2021023).

3. Results

The ages of the 16 male workers who worked in the manufacturing industry ranged from 18 years to 50 years. The ages in the subjects were shown in Table 1. The workers included nine-day shift workers and seven rotating night shift workers. Among the rotating night shift workers, 5 workers had worked the night shift for less than 5 years, one worker had worked the night shift for 10–15 years and one worker had worked the night shift for more than 20 years.

Table 1. Characteristics of participants

		Day shift workers (n=9)	Rotating night shift workers (n=7)
Age	≦20	1	0
	21-30	4	2
	31-40	1	2
	41-50	3	3
Assessment of sleep	**Sleeping hours (hours)**		
	<5	0	2
	5-6	7	2
	6-7	2	2
	unclear	0	1
	Sleep disturbance (Multiple answers allowed)		
	No	3	1
	Difficulty in getting to sleep	3	2
	No feeling of deep sleep	5	2
	Interrupted sleep	0	1
	Early morning awakening	0	2

3.1 Hair Cortisol Concentration

As can be seen in Table 2, the mean (± standard deviation: SD) HCC in the 16 workers was 17.28 ± 7.39 pg/mg and the median HCC was 16.86 pg/mg. There was no significant difference between HCCs in day shift workers (17.98 ± 3.03 pg/mg) and rotating night shift workers (16.37 ± 1.86 pg/mg) (p = 0.659) (Figure 1). There was also no significant correlation between age and HCC (r = -0.302, p = 0.273).

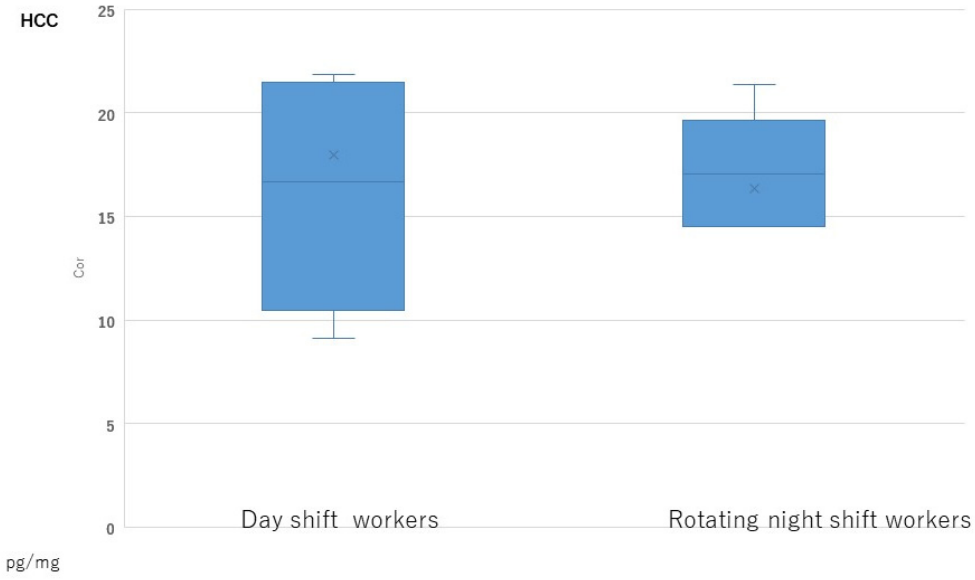

Figure 1. Hair cortisol concentrations in day shift workers and rotating night shift workers

3.2 Psychological Assessments

3.2.1 SRS-18

According to the assessment of psychological stress by SRS-18, one of the 16 workers had a definitely high level of stress, 5 workers had a slightly high level, 8 workers had a normal level and 2 workers had weak stress. In the 9-day shift workers, one worker had a definitely high level, 2 workers had a slightly high, 5 workers had a normal level and one worker had weak stress. Of the 7 rotating night shift workers, 3 workers had a slightly high level, 3 workers had a normal level and one worker had weak stress. Mean (± SD) SRS-18 scores were 15.67 (± 2.29) in day shift workers and 18.86 (± 6.03) in rotating night shift workers, and there was no significant difference ($p = 0.634$).

3.2.2 Job-Related Stress Scale

Median (25–75 percentile) job-related stress scale scores for job factors were 59 (50–66) in day shift workers and 62 (53-64) in rotating night shift workers. There was no significant difference between the two groups ($p = 0.791$). There were also no significant differences in 9 job-related stress factors (quantitative overload, qualitative overload, physical overload, job control, skill discretion, interpersonal relationships, workplace environment, job fitness, and satisfaction with work) between day shift workers and rotating night shift workers.

3.2.3 VAS

Median (25–75 percentile) VAS scores were 60 (45–75) in day shift workers and 60 (48–70) in rotating night shift workers. There was no significant difference between the two groups ($p = 0.669$).

Table 2. Stress assessment

			Day shift workers	**Rotating night shift workers**	**P value**
Biological	HCC	HCC	17.98 (+/- 3.03)	16.37 (+/- 1.86)	0.659
Psychological	SRS 18	SRS 18	15.67 (+/- 2.29)	18.86 (+/- 6.03)	0.634
	Job-related stress	Job factors	59(50-66)	62(53-64)	0.791
		Quantitative overload	6(4-9)	6(6-8)	0.824
		Qualitative overload	6(5.5-7.5)	7(6-8)	0.275
		Physical overload	3(1.5-3)	3(2-4)	0.288
		Interpersonal relationships	7(6.5-8)	7(7-8)	0.820
		Workplace environment	2(1-3)	2(2-3)	0.578
		Job control	7(5.5-9.5)	7(5-7)	0.551
		Skill discretion	2(2-3)	3(3-3)	0.078
		Job fitness	3(1.5-3)	2(2-2)	0.388
		Satisfaction with work	3(1-3)	2(2-2)	0.469
	VAS		60 (45-75)	60 (48-70)	0.669

3.2.4 Correlations of HCCs with Psychological Factors

There were no significant correlations of HCCs with job-related stress scale scores ($r = -0.115$, $p = 0.672$), SRS-18 scores ($r = -0.019$, $p = 0.944$), and VAS scores ($r = -0.135$, $p = 0.618$).

3.2.5 HCCs in Two Groups According to SRS-18 levels

We divided the workers into two groups according to SRS-18 levels (a group in which the stress condition was weak or normal and a group in which the stress condition was slightly strong or definitely strong) and we compared HCCs in the two groups. The median (25–75 percentile) HCC in the group in which the stress condition was weak or normal was 19.98 (16.13–21.50) pg/mg and the median HCC in the group in which the stress condition was

slightly strong or definitely strong was 12.77 (8.46–17.04) pg/mg. There was a significant difference in HCC between the two groups (p = 0.030) (Figure 2). In day shift workers, the median HCC in the group in which the stress condition was weak or normal was 18.29 pg/mg and the median HCC in the group in which the stress condition was slightly strong or definitely strong was 16.16 pg/mg. In rotating night shift workers, the median HCC in the group in which the stress condition was weak or normal was 20.59 pg/mg and the median HCC in the group in which the stress condition was slightly strong or definitely strong was 11.15 pg/mg.

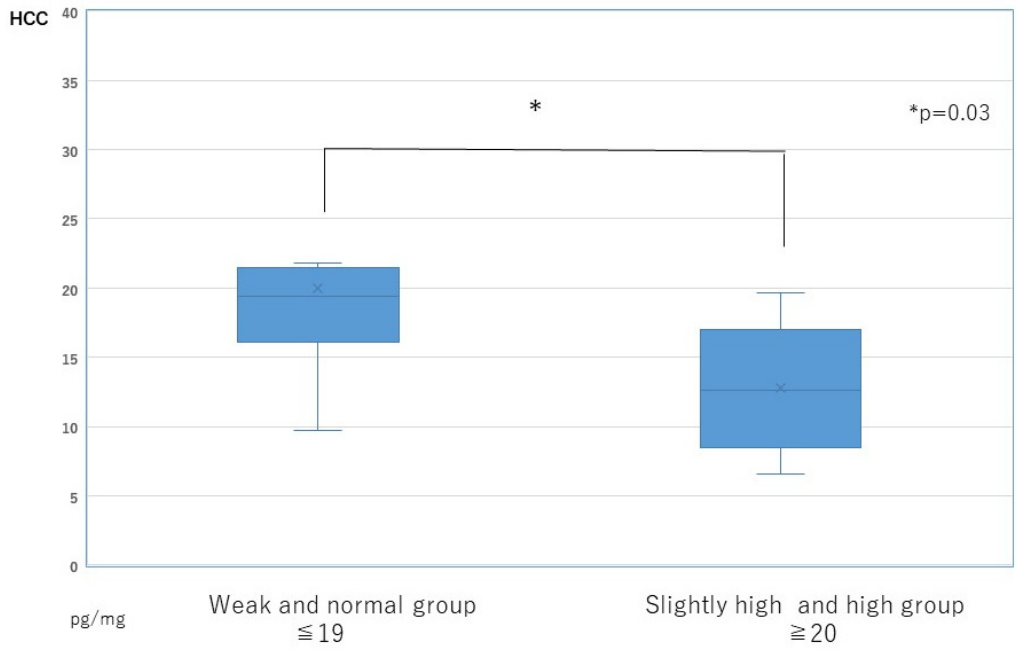

Figure 2. Hcc in two groups according to SRS18 Scores

3.2.6 Sleeping Hours and Quality of Sleep

Sleeping hours and sleep disturbance in the 9 day shift workers and 7 rotating night shift workers are shown in Table 1. There was no significant difference in sleeping hours between day shift workers and rotating night shift workers (p = 0.172). The proportion of workers with sleep disturbance was higher in rotating night shift workers than in day shift workers.

4. Discussion

Rotating night shift work is a possible source of work stress. However, in the present study, there were no significant differences in SRS-18 and job-related stress scores, which are psychological stress scales, between day shift workers and rotating night shift workers. In addition, we showed that there was no significant difference in HCC between day-shift workers and rotating night-shift workers. Results of previous studies in which HCCs were compared in day workers and shift workers were controversial. It has been reported that HCC was significantly higher in shift workers than in day workers but that there was no significant difference between HCCs in shift workers and day workers at an older age (Manenschijn, van Kruysbergen, de Jong, Koper, & van Rossum, 2011). Zhang et al. reported that HCCs in different shift workers (two shifts, three shifts and four shifts) were significantly higher than HCC in fixed day-shift workers (Zhang et al., 2020). However, Janssens et al. reported that HCC in shift workers was significantly lower than that in day workers after adjustment for age (Janssens et al., 2017). The reason of these different results may be the differences in subjects and study design.

The results for psychological stress scales and HCCs in the present study suggest that rotating night shift workers who have been working for a long time might not feel that they have strong stress. Rotating night shift workers who have the ability for adapting to night shift work might continue night shift work. Janssens et al. did not find a significant relationship between any of several job stressors and HCC and they found that HCC was significantly

lower in shift workers than in day workers. They suggested that a possible explanation may be the healthy worker effect and that workers whose tolerance to shift work are higher might have been selected for the study (Janssens et al., 2017). Manenschijn et al. suggest that older shift workers suffer less from stress or adjust better to shift work than younger shift workers, resulting in habituation to alterations in the hypothalamic-pituitary-adrenal axis. They also suggested that individuals who have a high tolerance for shift work can still work as shift workers at an older age, whereas individuals who can not adjust to shifting work may stop shift work at a younger age (Manenschijn, et al. 2011). We previously reported that rotating shift workers in their 50s who have worked for a period of 25–40 years have decided the time for eating and sleeping and have made sleep and rest a priority. And paid more attention to their healthcare than day workers. (Kubo, Yasui, Matsuura, & Tomotake, 2022) The ability for self-control and resilience for adapting to night shift work may be important factors for the continuation of work. Rotating shift workers who have worked for a long time might have high levels of health both physically and mentally. That might be the reason why there were no significant differences in psychological stress scales and HCCs between day shift workers and rotating night shift workers in the present study.

There has been increasing interest in clinical applications of HCC measurement. However, differences in methods for measurements of HCC may be one of the reasons for the controversial results of previous studies regarding HCCs in shift workers and day workers. Different methods including a luminescence immunoassay, radioimmunoassay, and LC-MS/MS were used for measurements of HCC in previous studies (Lanfear, Voegel, Binz, & Paul, 2020; Zhang et al., 2020). Since absolute values of HCC differ depending on the method used for measurement, simple comparisons cannot be made among previous studies. In order to detect even a slight difference between HCCs in day shift workers and rotating night shift workers, we used LC-MS/MS, which has high specificity. LC-MS/MS can directly measure the molecule of interest, but this method is expensive. In a review regarding a comparison of measurements using an enzyme-linked immunosorbent assay and LC-MS/MS, Greff et al. suggested that techniques need to be developed for better detection of hair cortisol using MS and for easier detection of hair cortisol without cross-reactivity (Greff et al., 2019).

Unexpectedly, HCC in the group in which the stress condition was slightly strong or definitely strong according to SRS-18 was significantly lower than HCC in the group in which the stress condition was weak or normal according to SRS-18. The reason for this might be that workers who feel strong daily stress can cope well with stressful conditions. It is possible that workers who feel strong daily stress can achieve a high level of resilience. For workers in whom the stress condition was normal or weak, there may be an accumulation of stress due to a long-term continuation of stress response even if the stress response is weak. Thus, workers with weak stress conditions might have a high HCC. Kanner et al. showed by regression analysis that everyday hassles might be a considerably better predictor than life events of psychological symptoms (Kanner, Coyne, Schaefer, & Lazarus, 1981). Results of HCC measurements in medical students during their internship suggested that stress level differs according to individual resilience (Mayer, Lopez-Duran, Sen, & Abelson, 2018).

Results of previous studies regarding the relationship between HCC and sleep have also been controversial. Zhang et al. reported that workers with high HCCs had a higher prevalence of sleep disorders than that workers with low or intermediate HCCs (Zhang et al., 2020). Feller et al. reported that HCC was positively related to daytime sleeping in older individuals (Feller et al., 2014). However, Lanfear et al. reported that there was no association between HCC and daytime sleeping in older adults (Lanfear et al., 2020). El Mlili et al. suggested that differences in sleep duration may be involved in these controversial results (Mlili, Ahabrach, & Cauli, 2021). Fekedulegn et al. reported that the prevalence of self-rated relatively bad or very bad sleep quality was higher in 180 urban police officers who had worked night shifts in the past month than in 81 urban police officers who had worked day-shifts (Fekedulegn et al., 2016). Although we could not find a relationship between HCC and sleep disorders, we confirmed that the prevalence of sleep disturbance was higher in night-shift rotating workers than in day shift workers. Thus, night-shift workers might have a poor quality sleep. Further study with a larger number of subjects is needed.

The small sample size was a limitation of the present study. Also, it is difficult to distinguish between job-related stress and non-job-related stress. Although the degree of physical fatigue in night shift workers was assessed in a previous study (Hu & Lodewijks, 2020), it is difficult to assess only stress related to night shift work. It has been suggested that elevated HPA activity can occur under a variety of conditions including physical exercise and metabolic abnormalities (Meyer et al., 2012). Thus, it should be kept in mind that HCC is influenced by such conditions.

5. Conclusion

In conclusion, there was no significant difference between HCCs in day shift workers and rotating night shift

workers. Day shift workers and rotating night shift workers have similar degrees of job-related stress. In rotating night shift workers who feel strong stress, the acquirement of resilience due to stress coping for medium- to long-term stress may be involved in low HCC.

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Competing Interests Statement

The authors declare that there are no competing conflicts of interest.

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