THE EFFECT OF NIGHT-SHIFT WORK ON THE LEVEL OF URINARY 6-SULPHATOXYMELATONIN IN MEN OVER FIVE DAYS

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Abstract

Night-shift work may adversely affect health. This study aimed to assess the effect of night-shift work on the level of urinary 6-sulphatoxymelatonin. Repeated measures of urinary 6-sulphatoxymelatonin on the morning before night-shift work and after first, third and fifth night-shift work was undertaken in 29 males, Malay, day-night-shift workers. A total of 29 male workers participated in this study. Median age was 29 years old. The highest level of urinary 6-sulphatoxymelatonin was at time point 1, followed by a significant reduction from time point 1 to time point 2. Subsequently, there was a progressive increase of urinary 6-sulphatoxymelatonin from time point 2 to 4. However, the urinary 6-sulphatoxymelatonin did not normalise at time point 4 and remained lower than the baseline reading taken at time point 1. This appears to suggest that workers were not able to achieve circadian adaptation to night-shift work even after five days of night-shift work. Findings from the current study could be useful as a reference, in developing strategies to enhance circadian adaptation to night-shift work.

Keywords: Circadian Rhythm, Melatonin, Night-shift, Shift Work, Sleep Quality

Introduction

Shift work refers to a work schedule that involves irregular or unusual hours, such as rotating shift work and night work, as compared to usual daytime work (1). As shift work is essential especially in manufacturing sectors to optimise productivity and maintain business competitiveness, it is widely adopted around the world (2).

Shift work has been found to be associated with a myriad of adverse health impacts including cardiovascular diseases, metabolic syndrome, cancers, and reproductive disorders (3-6). Furthermore, a number of studies have shown that night-shift work is associated with poor general wellbeing, as well as depression and anxiety (7, 8). Melatonin is believed to be an important marker of circadian rhythm linking night-shift work and circadian desynchronisation. It is thought that night-shift work reduces the secretion of melatonin given that production of melatonin takes place in the darkness and is inhibited by exposure to bright light at night (9). Previous studies have further demonstrated that decreased secretion of melatonin was associated with diabetes mellitus, cancers and mental health (10, 11).

Besides light exposure, it has been demonstrated that age, lifestyle characteristics, reproductive factors, and medication influence melatonin production. Age was found to be inversely associated with melatonin production(12). Smoking, alcohol and caffeine intake are examples of lifestyles factors that have been found to reduce melatonin production (12, 13). With regards to reproductive factors, melatonin was found to be lower among menopausal women (12). Finally, medication such as anti-depressants, sedatives, beta-blockers, non-steroidal anti-inflammatory drugs (NSAIDs) and hormone replacement therapy have been found to reduce melatonin production (14) and an increase in the level of melatonin with the use of oral contraceptives (15).

Given the scarcity of evidence and a high demand for night-shift work worldwide, more evidence is needed to improve our understanding on the impact of night-shift work on melatonin. Some studies have reported a decrease in the level of urinary 6-sulphatoxymelatonin (aMT6s), which is the metabolite of melatonin, during or after night-shift work (9, 16-18), whereas other studies have found an opposing

effect (12, 19, 20). Moreover, the pattern of change in the urinary 6-sulphatoxymelatonin levels over continuous days of night-shift work is not well known. Given that a high proportion of Malaysian workers are involved in night-shift work, it is important to improve our understanding of the adaptation mechanisms, as indicated by the changes in the urinary 6-sulphatoxymelatonin during night-shift work. Thus, this study aimed to assess the effect of night-shift work on the level of urinary 6-sulphatoxymelatonin. This information apart from serving as a reference for future research in this area, may be important in designing better shift work schedules and developing strategies to enhance circadian adaptation in night-shift work, to ensure healthier and productive workers.

Methods

Study design

This longitudinal study was conducted between 16th November to 22nd November 2016. Male manufacturing workers from a factory located in Klang Valley were invited to participate in this study. Invitation was conducted by having briefing between interested employees and author. Day-night-shift workers aged from 20 to 35 years old with no known medical illnesses were eligible for this study. Day-night-shift work schedule consisted of five days of daytime work (8.00 am - 8.00 pm), followed by five days of night-shift work (8.00 pm - 8.00 am) with two days off, and this cycle continued. Participants with history of primary sleep disorder such as sleep apnoea or recent history of using medications (Non-steroidal Anti-inflammatory Drugs [NSAIDS], anxiolytic, hypnosis) were excluded (12). These criteria were intended to ensure that participants were not under the influence of known factors that may alter levels of the melatonin production (12).

Sample size calculation

Sample size was calculated using the mean urinary 6-sulphatoxymelatonin with the significance level (α) preset at 0.05 and power of the study (1-β) as 80%. In a previous study the response within each subject group was normally distributed with standard deviation of two and the true difference between the night-shift workers and non-night- shift workers was 1.5 (19). The ratio of participants before and after night-shift work was taken as 1. The required sample size was 29 participants. Repeated measure of urine collection was performed on 29 day-night-shift workers for four days.

Data collection

The study was scheduled from the last day of their day-shift work to the fifth day of their nights-shift work. Participants completed a self-administered Malay language questionnaire on socio-demographic, lifestyle characteristics (smoking and alcohol intake) and occupational history prior to first urine sample collection. Participants were advised to empty their bladder before 3:00 a.m. and not to void urine within this period until urine collection. Spot urinary samples were collected between 5.30 am and 6.30 am using urine containers from participants at four different time points (days). First time point: morning during daytime work, second time point: at the end of first night-shift work, third time point: at the end of third night-shift work and fourth time point: at the end of fifth night-shift work. Participants were required to sleep during daytime after night shift ended. The main purpose for standardisation in the collection time was to minimise normal circadian variation in melatonin level (21). The collected samples were sent to the Department of Social and Preventive Medicine (SPM), University of Malaya on the same day and the urine was stored in freezer. According to manufacturer, storage of urine at temperature of 2-8 °C grants stability for four days. The samples were assayed within three days after urine sample collection. Urinary 6-sulphatoxymelatonin was measured using competitive enzyme-linked immunosorbent assay (ELISA) with a capture antibody technique, using the reagent from IBL (Germany). The assay was run in duplicated with low and high kit controls and it was carried out on a microtiter plate coated with anti-rabbit IgG. The intra-assay and inter-assay coefficient of variations were 5.2% to 12.2% and 5.1% to 14.9% respectively.

Statistical analysis

The collected data was analysed using the statistical programme SPSS version 20.0. Continuous variables were expressed as mean ± SD. Discrete variables were reported as frequency and percentage. Due to the non-normal distribution (skewed) of morning urinary 6-sulphatoxymelatonin concentrations, the concentrations were transformed to the logarithmically of the original values. General linear model for repeated measures were run to compare the mean of urinary 6-sulphatoxymelatonin concentrations at four different time points.

Results:

A total of 29 Malays workers from 1 manufacturing factory participated in the study, with the mean age of 27.1 ± 3.5 (Table 1). A majority (86.2%) of them were current smokers and most of them never consumed alcohol. The mean duration of night-shift work was 7.7 ± 2.9 years.

The geometric means (ng/ml) of urinary 6-sulphatoxymelatonin at four different time points is showed in Table 2. Generalised linear model (GLM) for repeated measures indicated that the level at time point 1 (baseline sleep) was the highest, with a significant reduction from the time point 1 to the time point 2 before there was a progressive increase from the time point 2 to the time point 4.

There was a significant reduction in the geometric means (ng/ml) of urinary 6-sulphatoxymelatonin level at the end of the first, third, and fifth nightshift (time points 2, 3 and

Table 1: Characteristics of study population

Characteristics	n (%)
Age (mean in years ±SD)	27.1±3.5
Ethnicity	
Malay	29 (100.0%)
Smoking status	
Current smoker	25 (86.2%)
Never smoker	4 (13.8%)
Alcohol intake	
Ever	1 (3.4%)
Never	28 (96.6%)
History of night-shift work	7.69±2.87
BMI (mean in kg/m²±SD)	27.10±3.42
Sleep quality	
Poor sleep quality	25 (86.2%)
Good sleep quality	4 (13.8%)

Table 2: Geometric means (ng/ml) of urinary 6-sulphatoxymelatonin (aMT6s) at four different time points

Time point	Mean morning aMT6s (ng/ml) (SE)	95% CI (ng/ml)
Time point 1 (night time sleep)	1.43 (0.02)	1.39-1.49
Time point 2 (at the end of first night-shift work)	0.95 (0.06)	0.83-1.07
Time point 3 (at the end of third night-shift work)	1.07 (0.04)	0.99-1.16
Time point 4 (at the end of fifth night-shift work)	1.11 (0.04)	1.02-1.19

4, respectively) relative to daytime work with night-time sleep (first time point 1) (Table 3). It was found that the concentration gradually and statistically increased from the second to fourth time points, with a significantly higher concentration at the end of the fifth nightshift (time point 4) than the concentration at the end of the first nightshift (time point 2).

Figure 1 shows that the highest level of geometric means (ng/ml) of urinary 6-sulphatoxymelatonin was at the time point 1, followed by a significant reduction from the time point 1 to the time point 2. Subsequently, there was a progressive increase of urinary 6-sulphatoxymelatonin over consecutive days of night-shift work (from time point 2 to time point 4). However, the urinary 6-sulphatoxymelatonin did not normalise at time point 4 and remained lower than the baseline reading taken at time point 1.

Table 3: Pair wise comparisons of geometric means of urinary 6-sulphatoxymelatonin (aMT6s) at four different time points

Measure: aMT6s						
(I) Time point	(J) Time point	Mean difference (I-J)	Standard error	*p*-value	95% CI for difference[b] Lower Bound	95% CI for difference[b] Upper Bound
1	2	0.49*	0.06	<0.001	0.32	0.65
	3	0.36*	0.04	<0.001	0.24	0.49
	4	0.33*	0.04	<0.001	0.21	0.44
2	1	-0.49*	0.06	<0.000	-0.65	-0.32
	3	-0.13	0.07	0.586	-0.33	0.08
	4	-0.16*	0.05	0.031	-0.32	-0.01
3	1	-0.36*	0.04	<0.001	-0.49	-0.24
	2	0.13	0.07	0.586	-0.08	0.33
	4	-0.04	0.07	1.000	-0.23	0.15
4	1	-0.33*	0.04	<0.001	-0.44	-0.21
	2	0.16*	0.05	0.031	0.01	0.32
	3	0.04	0.07	1.000	-0.15	0.23

Based on estimated marginal means
*. The mean difference is significant at the .05 level.
b. Adjustment for multiple comparisons: Bonferroni.

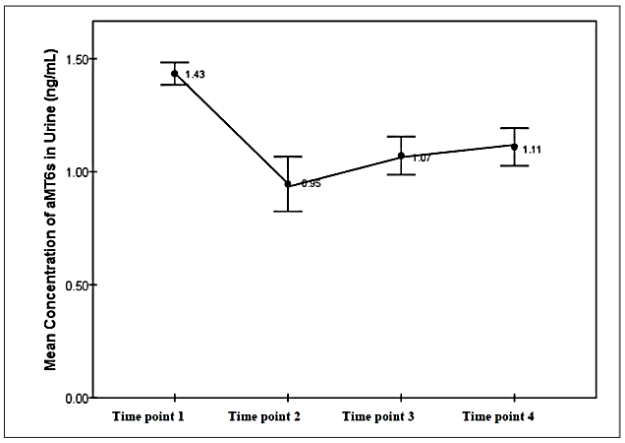

Figure 1: Changes of geometric means (ng/ml) of urinary 6-sulphatoxymelatonin (aMT6s) at four time points

Discussion

Urinary 6-sulphatoxymelatonin was collected in the current study because it has been shown to reflect the peak and approximately 70 per cent of the total nocturnal plasma melatonin secretion of the previous night (22, 23). Therefore, following the practice in previous epidemiological studies (12, 20, 24), it was used as an indicator of nocturnal melatonin production in the present study. Participants were required to empty their bladder two to three hours prior to urine collection to ensure

that the urinary 6-sulphatoxymelatonin in all the samples covered the same portion of the melatonin secretion curve (25).

The immediate reduction in the urinary 6-sulphatoxymelatonin level after the first night-shift work relative to night-time sleep suggests melatonin suppression due to the light-at-night effect. Melatonin secretion is found to be very sensitive to light exposure; illumination as low as 250 lux has been reported to delay the onset the melatonin secretion (26). The above finding is consistent with that of a few previous studies that have reported a significant reduction in the urinary 6-sulphatoxymelatonin level after night-shift work, and which have posited that this reduction is due to the effect of light at night (16, 17, 27). Experimental and epidemiological studies have shown an inverse association between light exposure and melatonin level, where an increased level of light exposure is associated with decreased melatonin production (17, 28).

Our results also showed that while the level of urinary 6-sulphatoxymelatonin among night-shift workers remained low compared to the night-time sleep level, it gradually increased over five consecutive night shifts. The increasing trend in urinary 6-sulphatoxymelatonin production over time most likely indicates that the participants were not able to adapt to night-shift work because the melatonin excretion rhythm was not adjusted to night-shift work. However, some previous studies have shown that there is a progressive decline in night-time melatonin over consecutive night-shifts, which reflects the circadian disruption was associated with the process of re-entrainment after consecutive night-shifts (18, 29). These conflicting findings possibly indicate that the speed of adaptation to night-shift work between Western and Malay populations may differ due to differences in genetic factors, hormonal levels, body size, or lifestyle habits that might influence the melatonin level (14, 15, 27, 30, 31). It should be noted that, at present, there is insufficient information on the adaptation of night-shift workers to night-shift work among Malay populations, thus a comparison cannot be made between the Malay population and other studies in the region.

Besides being an indication of poor adaptation, the increasing trend in urinary 6-sulphatoxymelatonin production from the time point 2 to the time point 4 could be due to the effect of progressive phase delay, where the peak of melatonin secretion (normally around 2.00 am to 4.00 am) is shifted to a few hours later (6.00 am to 8.00 am or even later) (32). It has even been found that the peak of melatonin secretion happens after two to three hours during daytime sleep when there is complete adaptation (33). In the present work, it is nonetheless conceivable that participants might have voided urine containing the peak of nocturnal melatonin secretion (when phase delay might not yet have occurred) before the actual urine collection (5.30 am to 6.30 am). Normally, phase delay happens after multiple night in a row, in which the central sleep-wake cycle adapted to being awake at night and lead to delay in the time of maximum secretion of urinary 6-sulphatoxymelatonin (29). Thus, the increased level of urinary 6-sulphatoxymelatonin at the end of the fifth nightshift may be explained by the effect of phase delay, when the peak of nocturnal melatonin secretion was captured, a hypothesis that accords with previous studies that have reported that phase delay (adaptation) seldom happens during the first three days of night-shift work (29, 34). Moreover, the timing of peak melatonin production can vary from one individual to another, as demonstrated in a previous study that found evidence for a large variation between individuals (35). The speed of phase delay among the Malay population remains to be elucidated due to the current scarcity of data.

Other possible factors that may have led to the increase in melatonin with the progression of night-shift work in the current study could include poor sleep quality and short sleep duration during daytime. Difficulty sleeping in the daytime has been identified as the main factor of intolerance to night-shift work (25). Various factors have been found to affect night-shift workers' daytime sleep quality. The main reason is possibly due to poor circadian adaptation where there is no increase in the proportion of melatonin secretion during daytime sleep after continuous night-shift work (33). Circadian adaptation is considered when greater proportion of the peak of melatonin secretion happens during the daytime sleep, which is associated with a better daytime sleep quality (33). However, melatonin secretion during daytime sleep may be partially suppressed by light if the individual does not sleep in a dark room during daytime (36). One laboratory study reported that the concentration of urinary 6-sulphatoxymelatonin for the first three days of daytime sleep is lower than that of the baseline sleep, but there is no difference in urinary 6-sulphatoxymelatonin concentrations between day sleep from the fourth day onwards and that of baseline sleep (34). The increased level of melatonin from the time point 2 to time point 4 indicates that the participants have become gradually adapted to being awake at night and sleeping during the daytime (34). Due to poor circadian adaptation and possibly poor daytime sleep quality, individuals may take naps during the last few days of night-shift work in order to alleviate sleepiness (25). Naps during night-time may influence melatonin secretion, which could be one of the factors that caused a slight increase in the urinary 6-sulphatoxymelatonin level on the last (fifth) nightshift (time point 4) in the current study.

We acknowledge some limitations of the present study. The study design limited the comparison of urinary 6-sulphatoxymelatonin between night-shift workers and day-shift workers. The day-shift worker rather than the day-night-shift worker is a better a proxy for night-time baseline sleep when doing daytime work. Another limitation of this study is that urinary 6-sulphatoxymelatonin was determined by using a spot urine sample. The use of a single morning urine sample may lead to an underestimation of the total melatonin secretion because previous studies

have shown that the timing and duration of the period of urine melatonin production among workers on night-shift work can change (36). Moreover, the concentration of urinary 6-sulphatoxymelatonin in the present study was not adjusted for urinary creatinine concentration, which would have allowed for adjustment of differences in urine concentration. It is further acknowledged that the level of light, either in the workplace or in the sleeping area, was not measured in the present work. There is a possibility that the level of urinary 6-sulphatoxymelatonin varies due to variations in the duration or intensity of light exposure during each night-shift work. Nevertheless, any such effect is believed to be minimal because the participants did not change their work station and the scope of their job remained similar throughout the study period.

The novelty of our present work lies in the same participants were measured four times, with one urine sample collection on the morning of the day shift and a collection at the end of the first, third, and fifth night-shifts. As these samples were collected from the same individuals, the present study design allowed control over inter-individual differences in melatonin production and excretion (37). The stringent criterion for selecting participants for the investigation on melatonin is also a notable strength of the present study. Moreover, the collection of urine was fixed between 5.30 am and 6.30 am in order to minimise the effect of natural circadian variation on melatonin production (37). Lastly, the investigation on melatonin was only conducted among the male participants. To date, most investigations on melatonin have concentrated on female study samples due to the link between shift work and breast cancer (20), and few studies on melatonin have been undertaken in males.

The findings of the present study underscore the changes that occur in the level of urinary 6-sulphatoxymelatonin over continuous night-shift work, which could serve as a reference for future research in this area, especially in designing and developing strategies to enhance circadian adaptation to night-shift work. The suggestion that night-shift workers have a fixed bedtime sleep during the daytime on each day after night-shift work (38), while being exposed to bright light during night-time work and have minimised sunlight exposure during the morning commute to home might be useful in accelerating circadian adaptation (39). Reforms to labour policy, including modifications to night-shift work such as setting the maximum number of night-shift in a week or month, and the provision of adequate rest periods between nightshift may also be necessary to promote the well-being of night-shift workers.

Future studies aiming to analyse urinary 6-sulphatoxymelatonin concentration from every void during night-shift work are warranted. This would reveal the onset, peak, and offset of melatonin production. In turn, this would improve understanding of circadian disruption and the adaptation of workers tonight-shift work. In addition, it would be beneficial to find a way to discover the effect of night-shift work on the 24-hour production of melatonin because the spot collection of urine and the collection of urine during night-shift work might produce results that underestimate the total melatonin produced by night-shift workers. Researchers could also consider using an objective measurement of light intensity. Although light at night is often hypothesised to be an underlying mechanism in the association between shift work and diseases such as cancer and metabolic disorder, there are very few published epidemiological studies that have used an objective light measurement (19, 40). An objective light measurement could further clarify the role of night-time light in melatonin production. In the meantime, studies to find solutions to increase tolerance to shift work should be continued. With this regard, it is worth elucidating the role of interventions such as regulation of light in the workplace and darkness during daytime sleep, melatonin supplementation during daytime sleep, and strategic planning of naps including prophylactic nap (before the night-shift work) or recuperative nap (after the night-shift work).

Conclusion

This appears to suggest that workers were not able to achieve circadian adaptation to night-shift work even after five days of night-shift work. Findings from the current study could be useful as a reference, in developing strategies to enhance circadian adaptation to night-shift work.

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Ethical approval

Ethical approval was obtained from Medical Ethics Committee of University Malaya Medical Centre (MECID. NO: 20154-1234).

Conflict of interest

The authors declare no conflict of interest.

Informed consent

Written informed consent was obtained from all individual participants in the study.

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