

A study of pattern of secretion of salivary melatonin in shift workers

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Abstract

Background: Globalization and industrialization lead round the clock service providers (shift workers) vulnerable for circadian rhythm sleep disorders

Aim & Objective: To know how effect of shift work on secretory pattern of melatonin in saliva since there is growing evidence that antioxidant characteristic of melatonin may be reason for development of cancer in long standing shift workers in future.

Materials & Method: The study was observational, controlled, parallel study carried out in 30 shift workers (mean [SD] age: 21.33 [0.48] years) and 30 age matched healthy subjects (mean [SD] age: 24.97 [2.55] years). Saliva samples for the measurement of melatonin totally were collected from participants resting in dim light at 2 hr min intervals between 20:00 and 00:00 h.

Result: A decreasing time line trend of salivary secretion of melatonin was observed for shift workers.

Conclusion: The circadian phase is altered in shift workers as compared to day workers using salivary melatonin concentration as a marker of the circadian phase.

Keywords: shift work, melatonin, circadian phase

1. Introduction

Sleep is essential for survival and good wellbeing, but necessity of sleep and what sleep does to our wellbeing is not fully known. Sleeping hours for an individual may vary widely, usually from 6 to 10 hours a day. Nearly all people sleep at night [1]. Table 1 shows the variation in duration sleep depending upon age.

Table 1: Shows the variation in duration sleep depending upon age

Age and condition	Sleep Needs
Newborns (0–2 months)	12 to 18 hours
Infants (3–11 months)	14 to 15 hours
Toddlers (1–3 years)	12 to 14 hours
Preschoolers (3–5 years)	11 to 13 hours
School-age children (5–10 years)	10 to 11 hours
Adolescents (10–17 years)	8.5 to 9.25 hours
Adults, including elderly	7 to 9 hours

An endocrine hormone related to sleep which was thought to maintain biological clock in human being is melatonin. The pineal gland situated in the posterior end of epithalamus predominantly secretes melatonin. The melatonin signal forms a basis for

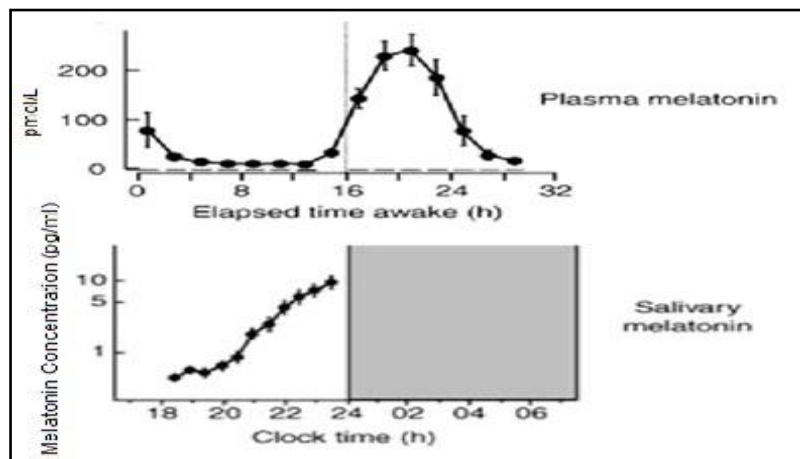
circadian cycle. Physiologically, melatonin causes drowsiness.

‘Production of melatonin by the pineal gland is inhibited by light and permitted by darkness as shown in above flow chart. It has been called "the hormone of darkness" and its onset each evening is called the Dim-Light Melatonin Onset (DLMO)’. Secretion of melatonin and its level in the blood peaks at the middle of the night gradually falls during the later part of the night with normal variations at other times according to an individual's daily sleeping habits.

Melatonin level in plasma is found to range from 30 to 250 pg/ml (table 2 and figure 1). Melatonin level progressively declines as the age advances as shown in the table. This hormone also exhibits diurnal variation i.e. its level raises towards the end of the day. Besides its primary function as synchronizer of the biological clock, melatonin may exert a powerful anti-oxidant activity. Melatonin is a powerful antioxidant that can easily cross cell membranes and the blood-brain barrier. This property acts to inhibit cancer [2,3].

Table 2: Time Courses of subjective sleepiness as assessed on the karolinska sleepiness scale plasma melatonin adapted with permission from Cajochen et al

Age	Plasma levels
1-3 years	250pg/ml
8-15 years	120 pg/ml
20-27 years	70 pg/ml
67-84 years	30 pg/ml

Figure 1: Time courses of subjective sleepiness (for information on the Karolinska sleepiness scale), salivary melatonin in a baseline 7.5 hrs constant routine followed by a 7.5 hrs sleep episode. Continuously measured data are plotted in 30 min bins. Adopted from permission from Kra Uchi et al

Economic and social requirements are now forcing the construction of a 24-hour society coupled with both economic profit and possible risk of health.

Shift work is highly prevalent in mechanized societies. An alteration in the sleep wake cycle produced by the dislocation of sleep to the daytime and work to the night time will impede with the circadian rhythm and homeostatic control of sleep. Shift work with deviant sleep schedule imposes a stress on the regular circadian rhythm and the exact margin between a normal response and a pathological response to this circadian stress remains unclear. [4,5] A night shift worker who sleep at the day time generally sleeps 1.5 to 2 hours less than day shift workers who sleep at night. It has been revealed that noise stimuli, even if it does not wake up one from sleep, has an impression on one's sleep cycle. The night shift workers have got to deal with sleep deprivation in addition to the effect of the disturbances in the circadian rhythm. Due to this, it is difficult for a shift worker to keep away from feeling tired.

American Academy of Sleep Medicine states that Shift-work sleep disorder (SWSD) consists of insomnia or excessive sleepiness. Most of the medical disability due to shift work occurs in night or early morning shift workers. The objective of this present study is to find the prevalence of Shift Work Sleep Disorder, amplitude of pattern of melatonin secretion in saliva during onset of night hours, widely known as

Dim Light Melatonin Onset (DLMO) in rotational shift workers and comparing the same parameters with age and sex matched day shift workers.

2. Materials and Methods

The study was conducted at the Institute of Physiology and Experimental Medicine, Madras Medical College after getting approval from Institutional Ethics Committee (IEC), Madras Medical College Chennai.

Thirty male subjects of age group 20 to 40 years on rotational shift with working hours other than 7 am to 7 pm, duration of not less than 6 months were selected. Thirty normal age matched day shift workers working on 7 am to 7 pm basis were taken as controls. Patient with conditions like were obstructive sleep disorder, psychiatric illness, on sleep medications, subjects with neoplastic, hepatic, diabetic, respiratory and any cardiovascular disorder or other concurrent medical illness were excluded from the study.

2.1 Estimation of Salivary Levels of Melatonin

2.1.1 Collection of saliva

The participants are given instructions for collection of saliva in sterile tube along with sterile cotton swabs. All the 30 night workers agreed to sample their saliva during working hours. They were instructed to collect saliva samples at 8 pm, 10 pm, 12 am with light level maintained constantly dim < 10 lux, such that endogenous secretion of melatonin was not suppressed. The samples collected were

transported and maintained at -20oC until further processing. Then they were shipped frozen to Tamil Nadu Dr.MGR Medical University for estimation of melatonin levels determined by ELISA reader. The estimation was done using Human melatonin ELISA kit CSB-E08132h Cusabio Biotech Co., LTD. Dongou Hi-tech Development Area, Wuhan, Hubein province, P.R China.

2.2 Statistical Analysis

The data collected were subjected to Statistical analysis using the Software SPSS version 21. Student’s t test was carried out to compare the means of variables between day worker sand shift workers.

3. Results

Melatonin secretary pattern in saliva showed a lower trend for night shift worker as evidenced by salivary melatonin ELISA assay. The night shift worker showed the following mean ± SD values at 8pm 3.77±0.56, at 10 pm 4.89±0.66, at 12 am 5.83 ± 0.25 (in pg/ml) as compared to day worker at 8pm 3.97 ±0.42, at 10pm 5.74± 0. 34, at 12 am 6.85±0.18 (in pg/ml). There was no significant difference in the melatonin level at 8 pm between night shift and day worker. But melatonin levels at 10 pm and 12 am were found to be statistically significant (p < 0.001) (Table 3 & 4 and Figure 2 & 3).

Figure 2: Comparison of parameters between Day worker and Shift worker

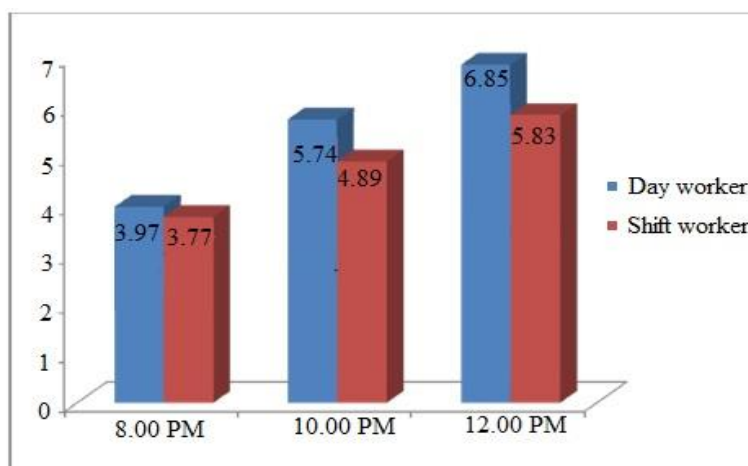


Table 3: Comparison of parameters between Day worker and Shift worker

Variable	Group	N	Mean
Age	Day worker	30	21.33±0.48
	Shift worker	30	24.97±2.55
BMI	Day worker	30	22.81±1.65
	Shift worker	30	24.04±1.64

Figure 3: Comparison of salivary melatonin level between day and Shift worker

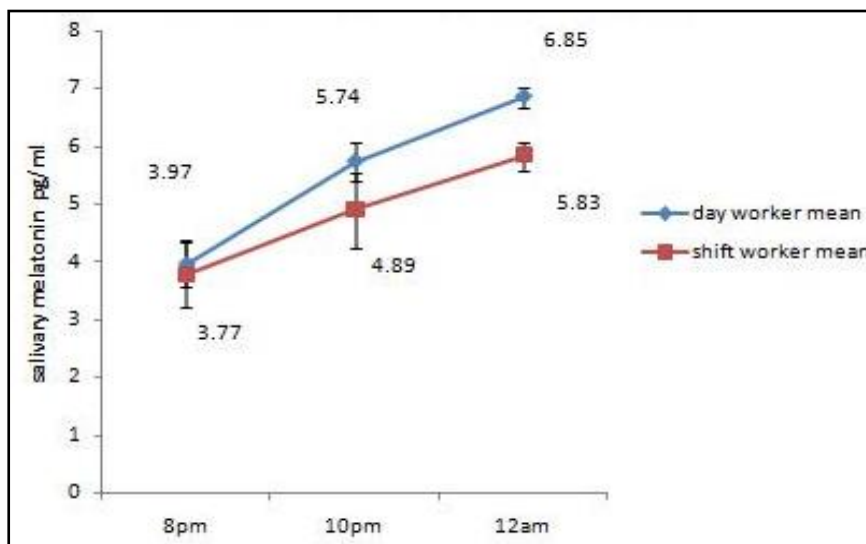


Table 4: Comparison of salivary melatonin level (pg/ml) between Day worker and Shift worker

Salivary melatonin	Worker	Number	Mean	SD	P-Value
Melatonin	Day	15	3.97	0.42	0.295
8 pm	Shift	15	3.77	0.56	
Melatonin	Day	15	5.74	0.34	0.000**
10PM	Shift	15	4.89	0.66	
Melatonin	Day	15	6.85	0.18	0.000**
12 am	Shift	15	5.83	0.25	

4. Discussion

Melatonin levels in those working rotational shifts were abnormal, with low levels. This pattern possibly suggests that shifts frequently changed (6 days, 6 nights, a day off between switch over) might not allow sufficient time for adaptation to occur. The consequences of this are unheard of.

So it is unclear from this study whether rotational shift workers actually produce less melatonin as a result of exposure to light-at-night, or just at different times. It is also unknown whether exposure to melatonin at different times results in different physiologic effects. Our study was conducted with rotational male shift workers, who might be more compliant than their female counterpart and the average population, so response and acceptability may vary in other work-related groups.

Comparisons of self-reported data and measured data did not support the utility of asking shift workers about bedroom lighting, but did support a possible association of duration of shift work with exposure to light at night and abnormal melatonin levels. Self-reported data can in certain circumstances be more valid than exposure measurements especially when it is measured at only one point of time because self-reporting may take into account the average exposure rather than taking a single snapshot. [6]

Accordingly, the bedroom lighting question could perhaps be improved to be more inclusive of the different conditions relevant to shift work, and then may show a closer association with measured light. Our observations must be interpreted cautiously, in light of two limitations: the size of the study and the use of a 3-sample measurement of salivary melatonin. In this study, we cannot rule out chances of potential confounders such as BMI, alcohol intake or co morbidities as alternate explanations.

5. Conclusion

The limitation of the study is base line secretion of melatonin prior to time 20:00 and melatonin concentration after time 00:00 were not measured. Therefore DLMO that occurred beyond this interval could have been missed. A study done by Gooneratne *et al* [7] stated that mean DLMO was

21:00 under strict dim light conditions in eighty-five subjects. The difference in time line trend of melatonin secretion between the day and night shift workers during dim light may be associated with disrupted circadian rhythm in rotational shift workers, which are further supported by the melatonin results.

Overnight or first-void urine specimens are also widely used although reliable for information on overnight melatonin production [8,9], they do not provide information on the 24-hour variation in melatonin levels. For the purposes of this study, multiple samples of saliva was considered as a simple and consistent measure of daily patterns of melatonin levels [10,11], which was more practical than repeated urine or serum sampling in occupational and residential settings. Even in the elderly, where hypo salivation and lower melatonin levels limit feasibility, salivary melatonin assessment was correlated with serum melatonin (Spearman rho = 0.659, $P = 0.001$). [7] More measurement time-points are desirable, and often feasible in laboratory studies, but in the field, the practical issue of compliance also was a consideration when selecting three sample times.

Few studies have attempted to measure light exposure directly for an extended period of time, or correlate such measurements with surrogate variables such as self reported shift work or self-reported home lighting levels, though in one study, melatonin was studied in night workers over four consecutive weeks [14]. Koller *et al* [15] measured light exposure in permanent night-shift workers for a period of 48 hours, while Dumont *et al* 2001[14] did measurements of light exposure and urinary 6-sulfatoxymelatonin in permanent night shift nurses for a period of 56 hours, followed by 24 hours in a laboratory setting. Dumont and colleagues [14] reported that 22 of their 30 permanent night nurses had a timing of melatonin secretion typical of day-oriented people, and that the 24-hour profiles of light exposure were very distinctive, similar to our observations in rotating shift workers. A cross-sectional study in US of health and performance markers in 188 day and night shift health care workers, Demoss C *et al* [15] reported an association with night shift work and unpredictable schedules, decreased energy levels, sleep disturbance and difficulty performing routine orders because of

sleepiness or fatigue. High waking and low sleeping melatonin could partly explain the results seen by DeMoss *et al* 2004.[15] They demonstrated that larger measurement studies are feasible and rotational shift work may be associated with abnormal melatonin levels and irregular light exposure patterns. So its use as a surrogate is supported by the above data.

Shift work is related to possible differences in light-at-night exposure, and may be a valid surrogate measure, but this needs to be confirmed with larger studies, wherein it will be important to look at both rotational shift and night-shift work. The high waking melatonin levels may contribute to problems of fatigue and alertness, which might be addressed by interventions such as exercise [16], or exposure to bright light during work and wearing dark glasses after work to improve adaptation to shift work [17]. The low levels of melatonin during sleep may be addressable with exogenous melatonin [18], though no long-term safety, dosing or formulation data exist at present.

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