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Original Research Article

Altered Circadian Secretion of Salivary Cortisol during Night Shift

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ABSTRACT

Background: Rotating night shift disrupts the circadian rhythms and has been associated with physiological stress, general fatigue, sleep disturbances and cardiovascular diseases. Alterations in the sleep pattern leads to increased cortisol and decreased melatonin which might be associated physiological stress and cardiovascular diseases.
Objectives: The objective of the present study was to investigate circadian secretion of salivary cortisol in rotating night shift nursing professionals.
Methods: 62 healthy nursing professionals of both genders performing day and night shifts duties were recruited. Each study subject had a monthly scheduled of regular 9 night shifts (12 hours night shift, from 20:00 to 08:00) followed by remaining 17-18 day shifts (6 hours day shift, from 08:00 to 14:00) with a total of 4 days off in between. Saliva samples were collected at around 8 hours interval while they were performing night duties and repeated when they were assigned day duties. Salivary cortisol level was estimated by the ELISA (Competitive) method.
Results: Significant difference was found in night cortisol among night (4.34 ± 3.37) vs day shift (2.70 ± 2.32), ($p<0.001$). Alteration in morning cortisol was also found between night (3.73 ± 2.47) vs day shift (5.00 ± 2.73). The circadian pattern of cortisol was altered by rotating night shift particularly at night and in the morning time.
Conclusion: The present study concluded that the desynchronization was appeared during night shift and entrainment of circadian rhythm was occurred in day shift.
Key words: *Rotating night shift; circadian rhythm; salivary cortisol.*

INTRODUCTION

Rotating night shift disrupts the circadian rhythms and has been associated with physiological stress, fatigue, sleep disturbances and cardiovascular diseases. Alterations in sleep pattern leads to increase cortisol and decrease melatonin which might be associated with sleep disorders, general fatigue syndrome, physiological stress and cardiovascular diseases. Those who work in night shift may attempt to sleep when their body clock is adjusted for the awakening phase.[1] This attempt disturbs the body clock resulting in a contradictory

relationship between sleep time and circadian schedule. It is possible that the circadian sleep propensity rhythm and hormonal rhythm are under influence of circadian pacemaker as well as sleep habit.[2] In developed counties, at least 15-20 % of the working population is engaged in shift work.[3]

Majority of the circadian rhythms in our body have both an endogenous component regulated by an internal clock, viz. the suprachiasmatic nuclei (SCN), and it synchronized with an exogenous component, light-dark cycle.[4,5] Disturbances in circadian rhythm may cause various physiological problems in shift workers especially where night shift is involved. In fact, long term shift work, rotating night shift work, has been linked to chronic stress with possible adverse consequences on the neuroendocrine system. [6]

Night shift work can affect cortisol secretion pattern which under normal secretion conditions, are high during day and low at night.[7] Cortisol is a essential hormone produced by the adrenal glands that affect various physiological functions, including metabolism and regulation of the immune system. Imbalances of cortisol are associated with fatigue, depression, obesity, immune dysfunction, diabetes and cardiovascular diseases.[7]

Salivary cortisol was estimated due to its stability in saliva for longer period and easy to take for circadian studies. The salivary cortisol concentration was synchronous with the serum concentration, indicating that the salivary assay could be substituted for the serum assay to assess circulatory rhythmicity across the 24-h time frame. Salivary cortisol appears to represent serum cortisol across the 24h period, except for those on oral contraceptives.[8] The more pronounced cortisol responses in saliva than in serum and its closer correlation with adreno-corticotropic hormone offer advantages over serum cortisol suggesting that salivary cortisol measurement may be used as an alternative parameter in dynamic endocrine test.[9] The salivary cortisol level were found to follow a profile similar to that of plasma, increasing significantly at each time point after CRH administration from their respective baseline values.[10] Another study shows that the salivary cortisol appears to be an excellent measure for monitoring circadian rhythm variation in adrenal activity in healthy individuals during shift work. [11]

The amount of cortisol present in serum and saliva undergoes diurnal variation, cortisol peaks in response to morning waking, declines gradually over the day, falls further after the onset of sleep and rises gradually in the early hours of the morning before waking.[12,13] Cortisol is secreted according to well defined circadian rhythm, and generally morning cortisol level increases rapidly following awakening with the peak level occurring 15-30 min after waking.[14] The present study was planned to investigate the circadian secretion of salivary cortisol in night shift nursing professionals and to find out whether they are reversible in due course of time.

MATERIALS AND METHODS

Subjects: Out of 82 volunteers, 20 were excluded due to non-fulfillment of study protocol. The duration and pattern of shift work were same among all the subjects, 62 healthy nursing professionals, aged 20-40 year, performing day and rotating night shift duties from 5-6 years and willing for compliance were randomly selected and recruited from Trauma Center, GM and Associated Hospitals, KGMU, Lucknow, UP, India. Each study subject had a monthly scheduled of regular 9 night shifts (12 hours night shift, from 20:00 to 08:00) followed by remaining 17-18 day shifts (6 hours day

shift, from 08:00 to 14:00) with a total of 4 days off in between. We recruited nurses of both genders from different wards and units viz. Intensive care unit (ICU), surgical emergency, Neurosurgery, Neurotrauma, Orthopedics emergency and Medicine emergency, who worked in rotating night shift. The study was approved by the institutional ethic committee (Ref. code: XXXIV ECM/B-P3), a detailed proforma was filled for all healthy nursing professionals, written informed consent was obtained from all the subjects willing to participate in the study. Subjects with any acute/chronic illness, known patients of diabetes mellitus, other endocrinal disorders, hypertension, coronary artery disease, subjects taking oral contraceptive pills and chronic renal diseases were excluded from this study.

Study design: The present prospective observational study was planned to investigate the circadian secretion of salivary cortisol in night shift nursing professionals and to find out whether they are reversible in due course of time.

Collection of Saliva and Urine samples: Saliva samples were collected at around 8 hours interval in their night shift duties (between 7-8 night out of nine night shifts) and day shift schedules (afternoon sample: between 13:00 to 15:00, night samples between 22:00 to 01:00 and morning samples between 05:00 to 08:00). The volunteers themselves collected the samples in different color vials. For collection of saliva samples, a notebook was provided to each subject with all details regarding the timing and procedure for sampling and their sleep-awake timing. All participants were instructed to wash their hands properly before taking the saliva samples and to rinse their mouth with water to remove food particles, if they had taken their meals. They were asked to refrain from eating or drinking anything for atleast 30 mints after awakening. Saliva samples were then centrifuged at 3000 rpm for 15 minutes. Saliva samples were frozen at -20^0C for 2 months and analyzed by the ELISA (Competitive ELISA) method.

Recording circadian pattern of Body Temperature: The subjects themselves recorded circadian pattern of body temperature (below the arm pit). For recording, a digital thermometer and notebook containing a fixed time interval of recording was provided to each subject. The timing pattern of body temperature recording was similar to that of collection of saliva samples. It was measured at every 8 hours by using Digital thermometer.

RESULTS

There were total 62 (32 male and 30 females) night shift nursing professionals recruited. The present study investigates the circadian secretion of salivary cortisol in night shift nursing professionals. All the data were summarized as Mean$\pm$ SD & baseline characteristics of male and female night shift workers are given in Table 1. Groups were compared by applying paired t test. A two tailed (α=2), $p<0.05$ was considered just significant, $p<0.01$ moderate/very significant and $p<0.001$ highly significant. P value elucidate that if it is < 0.05, < 0.01, < 0.001 then the null hypothesis would be rejected at 5 %, 1 % or 0.1 % respectively. Statistical analysis was carried out by using INSTAT 3.0 (Graph pad prism software; San Diego, CA).

In general, cortisol level rises in the early morning (just after awaking) and in the evening (reference range between 3-10 ng/ml). Afternoon cortisol level did not show a significant pattern between night (3.22 ± 2.09) vs day shift (2.97 ± 1.76). This pattern was closest to the reference range. Cortisol level was raised at night during night shift.

Table 1: Baseline characteristics of night shift workers.

Baseline Characteristics	Night Shift Workers (*n* = 62)
Age	24.74 ± 3.81
Weight (kg)	53.21 ± 8.85
Height (cm)	160.44 ± 8.16
Body mass index (BMI)	20.59 ± 2.40
Marital Status	
Married	16 (25.80%)
Unmarried	46 (74.19%)
Diet	
Vegetarian	23 (37.10%)
Non-Vegetarian	39 (62.90%)

Highly significant difference was found in night cortisol levels among night (4.34 ± 3.37) vs day shift (2.70 ± 2.32), ($p<0.001$) due to recovery during day shift.(Table 2) Alteration in mean morning cortisol level was also found between night (3.73 ± 2.47) vs day shift (5.00 ± 2.73) However, this pattern was moderately significant (Figure 1).

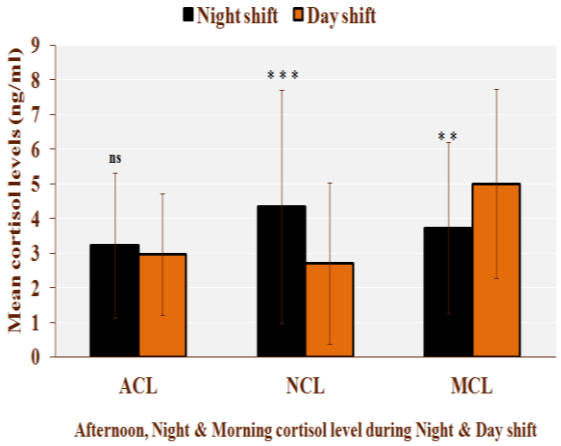

Figure 1: Mean Afternoon, Night and Morning cortisol levels during night and day shift (ACL: Afternoon Cortisol level; NCL: Night Cortisol level; MCL: Morning Cortisol level),(ns-$p>0.05$,**$p<0.01$, ***-$p<0.001$; I- Bar of the standard deviation).

Body temperature at night during night and day shift was as similar as the normal range. Insignificant pattern of body temperature was found at night and in the evening time between night vs day shift. ($p>0.05$) In morning, mean body temperature during night shift was 36.08 ± 0.71 and during day shift 36. 4 ± 0.67 ($p=0.01$) was statistically significant. (Figure 2)

Associations of salivary cortisol between different shift were done by Pearson correlation analysis. On the basis of sample size of this study, if $r=0.25$ then $p<0.05$ (significant), if $r=0.33$ then $p<0.01$ (moderate/very significant), if $r=0.41$ then $p<0.001$ (highly significant).

Afternoon and morning cortisol of night shift was positively correlated with that of day shift cortisol secretion (afternoon and morning). However this pattern was insignificant. ($p>0.05$) Highly significant and positive correlation was found in the pattern of night cortisol and night melatonin level between night and day shift. ($p<0.001$) (Table 2)

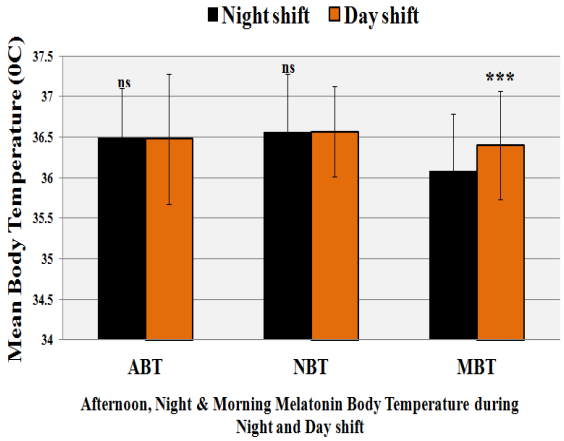

Figure 2: Mean Afternoon, Night and Morning body temperature during night and day shift (ABT: Afternoon Body Temperature; NBT: Night Body Temperature; MBT: Morning Body Temperature), ns-$p>0.05$, ***-$p<0.001$; I- Bar of the standard deviation).

Table 2: Correlation of Salivary Cortisol between Night versus Day shift.

Measured Variables	Night Shift (NS) versus Day Shift (DS)	r (n=62)
Salivary Cortisol	Afternoon cortisol: NS vs DS	0.12^{ns}
	Night Cortisol: NS vs DS	0.50***
	Morning Cortisol: NS vs DS	0.13^{ns}

$^{ns}p>0.05$ Not significant, ***$p<0.001$ highly significant.

DISCUSSION

In present study the increased level of cortisol at night during night shift might be associated with increased physiological stress and cardiovascular diseases. Cortisol secretion patterns may be impacted by night shift work, which could affect cardiovascular and cancer risk.[15,16] These findings reinforce previous studies which reported elevated exposure to cortisol on early shifts, relative to 'normal' later working days and rest days, might promote pathogenic processes including insulin resistance.[17] In other study, the related neurotransmitters like Urinary nor-epinephrine and Epinephrine were higher during work than non-work in the day, but in evening and night shift workers, the difference was small and in opposite direction. Working evening or night shift are independent predictors of Non –dipper status.[18] During night shift the hormones are more sensitive to endogenous components like catecholamine, prolactin, and growth hormones which showed an immune response to the shifted sleep/activity cycle, evidencing a "masking effect" due to the work activity. In another study, hormones having stronger endogenous components, such as cortisol and Melatonin, showed a more stable pattern, with a slight tendency for partial adjustment of cortisol during the second night.[19] It is also reported that the sleep factor (time of onset and/or period) seemed to be more potent in modifying the circadian rhythm of serum cortisol, especially with the night shift.[20] Present study exhibits the altered circadian secretion of salivary cortisol during night shift. Its secretion was higher at night time as compare to early morning during night shift. However recovery was found in day shift which shows the entrainment of circadian rhythm. These findings were somewhat similar with other studies shows the increased awakening cortisol level. The activation of hypothalamic pituitary adrenal (HPA) axis and the subsequent increase in cortisol secretion is a known physiological stress response.[21-23] however fewer studies have investigated the influence of long term night shift work on the HPA axis and the awakening cortisol.[24-26]

In the present study, subjects with rotating shifts of 12 hrs night works had complained of difficulty in sleep, decreased calculative tasks, impaired cognitive functions, decreased alertness, constipation, stress and mental fatigue which is in accordance with the other studies.[27,28] Quality and quantity of sleep are also affected by rotating night shift. Duration of sleep was shorter during day time at night shift (3-4 hours less as compare to night time sleep during day shift).

CONCLUSION

In present study, we concluded that rotating night shift altered the circadian secretion of cortisol particularly at night and in the morning time during night shift due to desynchronization. However, recovery (reversed in the normal range) was found when subjects went back to the day shift. Performing prolonged rotating shifts leads to increased cortisol level may be one factor contributing to an increased risk of cardiovascular diseases, physiological stress and other circadian rhythm disorders in night shift workers.

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Conflicts of interest: The authors declare that there is no conflict of interest.

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