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## Research Article

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### EFFECTS OF ROTATING NIGHT SHIFT AND EXPOSURE OF LIGHT AT NIGHT ON CIRCADIAN PATTERN OF SALIVARY CORTISOL AND URINARY MELATONIN LEVELS

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#### ABSTRACT

**Objectives:** The objective of the present study was to investigate the effects of rotating night shift and light exposure at night on circadian pattern of salivary cortisol and urinary melatonin levels.

**Methods:** 62 healthy nursing professionals of both genders performing day and night shifts (continuous 9 days night shift with alternate day shifts) were recruited. Each month scheduled to continuous 9 days night shift (12 hours in regular 9 nights, from 20:00 to 08:00); after 9 days night shift they perform remaining duties in day shift and 4 days off. Saliva and Urine samples were collected at around 8 hours interval while they were performing night duties and repeated when they were assigned day duties. Saliva and Urine samples were analyzed by the ELISA method.

**Results:** Significant difference was found in night cortisol among night ( $4.34 \pm 3.37$ ) vs day shift ( $2.70 \pm 2.32$ ), ( $p < 0.001$ ). Alteration in morning cortisol was also found between night ( $3.73 \pm 2.47$ ) vs day shift ( $5.00 \pm 2.73$ ). Night melatonin level was decreased as compared to morning melatonin. Significant difference were observed when compared night melatonin between night ( $16.71 \pm 11.98$ ) vs day shift ( $22.71 \pm 13.25$ ) ( $p < 0.001$ ), morning melatonin between night ( $20.07 \pm 14.13$ ) vs day shifts ( $28.26 \pm 14.14$ ) ( $p < 0.001$ ). The circadian pattern of cortisol and melatonin 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 in the day shift.

**Keywords:** Rotating Night shift; circadian rhythm; salivary cortisol; urinary melatonin.

#### INTRODUCTION

Rotating night shift disrupts the circadian rhythms and has been associated with fatigue, stress and sleep disturbances. Alterations in the sleep pattern leads to increased cortisol and decreased melatonin which might be associated with sleep disorders, anxiety, depression, general fatigue syndrome, stress and cancer risk. Those who work in night shift may attempt to sleep when their body clock is adjusted for the awakening phase.<sup>1</sup> 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.<sup>2</sup>

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.<sup>3,4</sup>

Melatonin is a hormone (N-acetyl 5 methoxy-tryptamine) synthesized and secreted principally by the pineal gland at night under normal environmental conditions. The pineal gland decides the amount of melatonin secretion based on information sent from the retina of the eye which contains a unique subset of cells that produce a pigment called melanopsin. Melanopsin allows a cell to detect light and dark. Information collected by the cells is sent along the retinohypothalamic tract (RHT), a sort of information highway that extend from the retina to hypothalamus. In hypothalamus, this information is transmitted to a cell called the suprachiasmatic nuclei (SCN). The SCN of the hypothalamus have melatonin receptors and melatonin may have a direct action on SCN to influence the circadian rhythm.<sup>5</sup> Melatonin secretion is enhanced in darkness and decreased by light exposure at night. Exposure to artificial light at night and disruption of the endogenous circadian rhythm with suppression of the melatonin synthesis has been suggested mechanisms.<sup>6</sup> Melatonin gives a measure of day length, it can reset the clock by acting as chemical zeitgeber. Melatonin is metabolized to 6-hydroxy-mel in the liver and the main metabolite excreted in urine is 6-Sulphatoxy-melatonin are more stable than 6-hydroxy melatonin in serum. The concentration of 6-Sulphatoxy melatonin or 6-hydroxyl melatonin sulphate in urine correlates with the total level of melatonin in the blood during the collection period. Melatonin levels in individuals with normal sleeping patterns begin to increase during the evening (~ 9:00 p.m.). Melatonin levels peak at around 2:00 a.m. and return to baseline around sunrise (~ 6:00 a.m.).<sup>7</sup> Irregular sleeping patterns can lead to circadian disruption and shift the amplitude and timing of peak melatonin levels.

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.<sup>8</sup> The more pronounced cortisol responses in saliva than in serum and its closer correlation with adreno-corticotrophic hormone offer advantages over serum cortisol suggesting that salivary

cortisol measurement may be used as an alternative parameter in dynamic endocrine test.<sup>9</sup> 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.<sup>10</sup> 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.<sup>11</sup> 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.<sup>12, 13</sup>

We are aware of the various circadian disorders caused by working in night shift but still we don't know their exact pathophysiology and their reversibility in due course of time. Our present knowledge explains the changes in circadian pattern on the basis of secretion of Melatonin, the chrono molecule and its ability to inter play with other hormones like Cortisol.

#### **Material & 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 (continuous 9 days night shift with alternate day shifts) from 5-6 years and willing for compliance were randomly selected and recruited from Trauma Center, GM and Associated Hospitals, KGMU, Lucknow, UP, India. Subjects were working in continuous 9 days light at night exposure in each month. 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 pattern of salivary cortisol and urinary melatonin levels in night shift workers and to find out correlation, if any, along with whether they are reversible in due course of time.

### Collection of Saliva and Urine samples

Salivary and Urine 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 and urine 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 and urine samples were frozen at -20°C 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 and urine 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. Salivary cortisol and Urinary melatonin (6-Sulfatoxy Melatonin) levels were analyzed in the present study. 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. Associations between variables were

done by Pearson correlation analysis. A two tailed ( $\alpha=2$ ),  $p<0.05$  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).

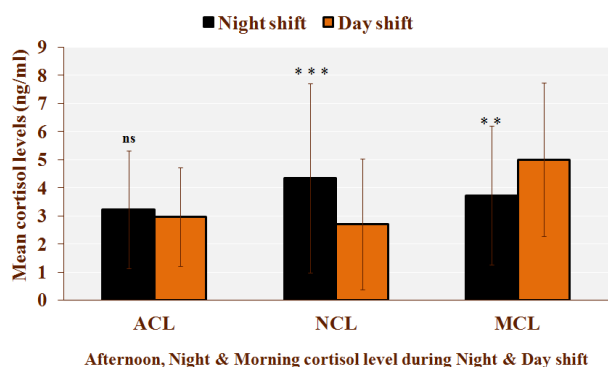
**Table 1: Baseline characteristics of night shift workers.**

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

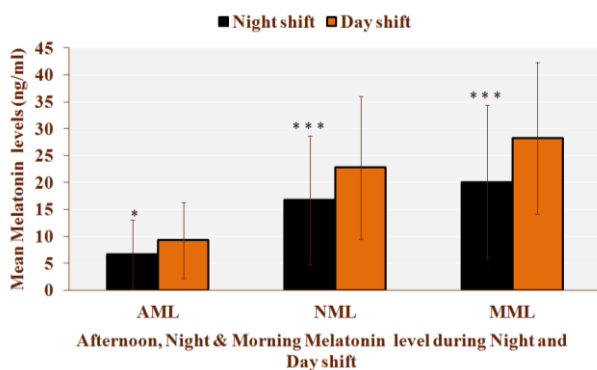
In general, cortisol level rises in the early morning (just after awaking) and in the evening (reference range between 3-10 ng/ml). Afternoon cortisol level did not show a significant pattern between night (3.22  $\pm$  2.09) vs day shift (2.97  $\pm$  1.76). This pattern was closest to the reference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

Highly significant difference was found in night cortisol levels among night (4.34  $\pm$  3.37) vs day shift (2.70  $\pm$  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  $\pm$  2.47) vs day shift (5.00  $\pm$  2.73) However, this pattern was moderately significant (Figure 1). Melatonin hormone also shows diurnal variation, its level increases from midnight to early morning and decreases in the late morning and in day hours. This pattern was found altered in rotating night shift workers during night. Melatonin synthesis directly depends upon transport of signal of light in the day time and conversion of serotonin into melatonin depends upon signals of darkness received at night. However, its level may differ from individual to individual.

**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- $p > 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ;  $\bar{x}$ - Bar of the standard deviation).



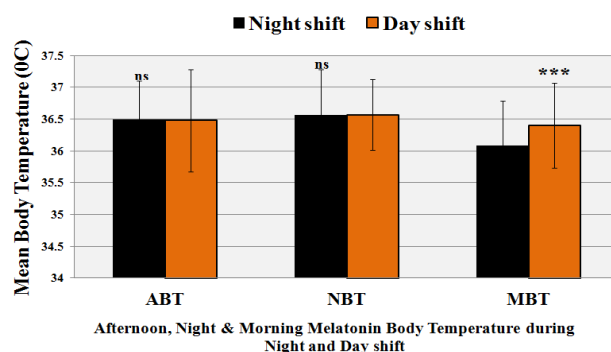
Normal range of melatonin is 0.8-40 ng/ml, its levels increases at midnight and declines in day time. Night melatonin level was found declined as compared to morning level and this pattern was significant when compared night melatonin between night ( $16.71 \pm 11.98$ ) vs day shift ( $22.71 \pm 13.25$ ) and morning melatonin level between night ( $20.07 \pm 14.13$ ) vs day shifts ( $28.26 \pm 14.14$ ) ( $p < 0.001$ ) (Figure 2). The result show that light exposure at night effect on melatonin production in rotating night shift. Altered melatonin levels were found in night and in the morning samples during night shift.



**Figure 2:** Mean Afternoon, Night and Morning melatonin during night and day shift (AML: Afternoon Melatonin level; NML: Night Melatonin level; MML: Morning Melatonin level), (\* $p < 0.05$ , \*\*\* $p < 0.001$ ;  $\bar{x}$ - 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  $36.08 \pm 0.71$  and during day shift  $36.4 \pm 0.67$  ( $p = 0.01$ ) was statistically significant. (Figure 3)



**Figure 3:** 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- $p > 0.05$ , \*\*\* $p < 0.001$ ;  $\bar{x}$ - Bar of the standard deviation).

Variables that were significant by paired t test and significantly affected due to night shift were correlated. Associations between variables 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).

Salivary cortisol and Urinary melatonin levels (Afternoon and Morning sample) of night shift was positively correlated with cortisol and melatonin level (afternoon and morning sample) of day shift. 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)

**Table 2:** Correlation of Salivary Cortisol and Urinary Melatonin between Night versus Day shift.

Measured Variables	Night Shift (NS) Vs. Day Shift (DS)	
	Comparison	r (n=62)
Salivary Cortisol	Afternoon cortisol: NS vs DS	0.12 <sup>ns</sup>
	Night Cortisol: NS vs DS	0.50 <sup>***</sup>
	Morning Cortisol: NS vs DS	0.13 <sup>ns</sup>
Sulfatoxy Melatonin	Afternoon Melatonin: NS vs DS	0.13 <sup>ns</sup>
	Night Melatonin: NS vs DS	0.51 <sup>***</sup>
	Morning Melatonin: NS vs DS	0.18 <sup>ns</sup>

<sup>ns</sup> $p > 0.05$  Not significant, <sup>\*\*\*</sup> $p < 0.001$  highly significant.

The correlation between salivary cortisol and urinary melatonin level in different shift (night and day shift) were summarized in Table 3. Moreover the correlation between afternoon, night and morning samples (cortisol and melatonin) in different shifts (night and day shift) were presented in Table 3. Generally, cortisol and melatonin hormonal levels should be negatively associated in normal individuals. At night time cortisol level should be decreased and melatonin level increased and in the day time cortisol level should be increased and melatonin level should be decreased.

**Table 3:** Correlation between Salivary Cortisol and Urinary Melatonin of Night versus Day shift.

Correlation between Cortisol and Melatonin	r (n=62)
Afternoon Sample (Night Shift)	0.02 <sup>ns</sup>
Afternoon Sample (Day Shift)	-0.04 <sup>ns</sup>
Night Sample (Night Shift)	-0.16 <sup>ns</sup>
Night Sample (Day Shift)	-0.30*
Morning Sample (Night Shift)	0.30*
Morning Sample (Day Shift)	0.08 <sup>ns</sup>

<sup>ns</sup>p>0.05 Not significant, \*p<0.05 just significant.

Afternoon cortisol and Afternoon melatonin level of day shift were insignificant and negatively associated while for night shift, afternoon cortisol and melatonin level were insignificant and positively associated. Night cortisol and night melatonin level of night shift were also insignificant and negatively associated while during day shift it was significant (p<0.05). Morning cortisol and morning melatonin of both night and day shift were positively associated. However, this association did not reach at significance level during day shift.

#### DISCUSSION:

Cortisol and Melatonin both hormones exhibit a diurnal variation in normal healthy individuals. Many studies have reported that subjects exposed to light and who remain awake at night have lower levels of melatonin at night, as melatonin synthesis always occurs at night, particularly in the darkness. Light exposure at night has been hypothesized as one of potential mechanisms of breast carcinogenesis in the night shift workers through inhibition of melatonin synthesis.<sup>6</sup> Findings of the recent study indicates that two nights of

rotating shift work may not change the timing of melatonin production to the day among those working at night.<sup>14</sup> While the results of the other study indicate that working eight or more night shifts per month may disrupt the synthesis of melatonin.<sup>6</sup> Finding of our study in agreement with previous study indicate that night shift workers have substantially lower 6-sulfatoxymelatonin during night work and daytime sleep, and levels remain low when night shift workers sleep at night. Chronic reduction in melatonin among night shift workers may be an important carcinogenic mechanism. Cortisol secretion patterns may be impacted by night shift work, which could affect cancer risk.<sup>15, 16</sup>

In present study the increased level of cortisol and decrease level of melatonin at night during night shift might be associated with risk of cancer. 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.<sup>17</sup> 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.<sup>18</sup> 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.<sup>19</sup> 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.<sup>20</sup> In night shift workers, sleep was initiated (on average) about three hours prior to the onset of melatonin production. In contrast, day-active subjects initiated sleep (on average) about three hours after their melatonin onset. Thus, the sleep times selected by night shift workers may not be well-synchronized to their melatonin rhythm, assumed to mark the phase of their underlying circadian pacemaker.<sup>21</sup> The change in the Acrophase of 6-sulfatoxy-melatonin was associated

with different shifts.<sup>22</sup> The overall advance of melatonin profile was primarily achieved during the initial exposure to an 8h period of darkness. The present data suggested that exposure to dark affects human circadian phase.<sup>23</sup> 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.<sup>24, 25</sup> 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 affects the circadian pattern of cortisol and melatonin 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. Prolonged exposure of light at night leads to increased cortisol and decreased melatonin level may be one factor contributing to an increased risk of cancer, cardiovascular diseases and other physiological rhythm disorders in rotating night shift workers.

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

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