# MELATONIN, CORTISOL, PROLACTIN AND IGA LEVELS AND THEIR IMPLICATION ON CIRCADIAN-BASED CANCER THEORY

By

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## Abstract:

**Introduction:** Shift work is a recognized stressful problem due to accumulation of mental and physical fatigue. It is a biological and biochemical process that begins in the brain and spreads through the autonomic nervous system causing hormone release and eventually exerting an effect on various body systems leading to health problems. Rotating shift work is associated with hypertension, cardiovascular troubles, fatigue, gastro-intestinal disturbance and poor sleep quality and may play a role in development of breast cancer. **Objective:** The current study aimed at assessment of different health hazards among rotating shift workers and the effect of shift work on cortisol, prolactin, and IgA levels with special reference to carcino-embryonic antigen (CEA). **Subjects and Methods:** The work was conducted on 46 female workers (nurses), matched with day shift female nurses as control group using a detailed questionnaire including occupational history. Clinical examination was performed. Cortisol, prolactin, melatonin, IgA and carcinoenbryonic antigen (CEA) serum levels were measured. **Results:** The prolactin and cortisol as well as CEA levels showed a statistically significant difference between rotating shift workers and the day-time workers. The melatonin and IgA levels were statistically significantly lower in rotating shift workers in contrast to those of day-shift workers. In correlation with the duration of employment and age of the worker, there was a statistically significant positive correlation with cortisol and CEA and a statistically significant negative correlation with melatonin. **Conclusion:** The study reported that shift workers had different health disorders including excess risk of development of breast cancer. **Recommendations:** CEA may be helpful as a prognostic tumor marker, so, further studies with CA125 and/ or CA 19-9 could be performed.
**Keywords:** Rotating  shift workers- CEA- Melatonin – circadian rhythm.

## Introduction

Sleep disruption, like that experienced by long-term rotating shift workers, is a physiological stressor which causes a variety of adverse physical, psychological and cognitive symptoms. Some cognitive symptoms are thought to be mediated by the direct effect of stress hormones on the hippocampus (Pavlis, 2007).

Regardless of its source, stress provokes endocrine responses in the body that affect the hypothalamic-pitutary-adrenal (HPA) axis. Acute activation of the HPA axis adaptively activates the body's stress response by increasing cortisol production.

Cortisol, which is a reliable indicator of stress, displays pronounced variation across the time-of-day with high levels in the morning and low in the evening. Stress may alter the secretion levels and circardian pattern of the hormone (Katia, 2008).

Long term increase of circulating cortisol or changes in the circadian rhythm of the hormone increase the risk of metabolic and cardiovascular disease (Akerstedt, 2003).

Rotating shift work is a stressful condition which interferes with the normal synchronization of body functions as well as with social habits. The resulting stress may result in higher prevalence of many diseases and in immune suppression which may, in turn, lead to reduced resistance to disease (Ng et al., 1999).

Several biological indices have been studied in the field of shift work e.g. melatonin, cortisol, catechocholamine and prolactin.

Prolactin which mirrors the dopaminergic activity in the brain seems to react to real life stressors in a systematic way. It is also influenced by sleep and tends to rise among rotators after nightshift (Weibel et al., 1999).

Melatonin is a hormone produced by the pineal gland in humans. It has a diverse range of physiological effects such as modulation of the sleep-wake, thermo-regulatory, cognitive, cardiovascular and immune systems (Dawson and Van Den Heuvel, 1998). The melatonin-generating system is characterized by three basic features: photosensitivity, circadian rhythmicity and age related decline in its activity.

Melatonin receptor regulates several second messengers: Cyclic adenosine monophosphate (CAMP), cyclic guanisine monophosphate (CGMP), diacylglycerdol, arachidonic acid and intracellular calcium concentrations (Vanecek, 1998).

Immunoglobulim A (IgA) is another potential biomarker for stress-induced immunologic effects in shift workers (Henningsen et al., 1992).

### Aim of the work

1.  To evaluate the disruption of the normal hormonal profile caused by rotating shift work.

2.  To study some health effects of night rotating shift work including the risk of development of hormone dependant cancer.

### Subjects and methods

The study included 46 shift workers in intensive care unit (ICU) of Kasr-El Ainy hospital and 20 morning – time workers from the administrative zone of the same hospital as controls.

The shift workers work under rotating three shift systems (7 am to 3 pm, 3pm to 11 pm and 11 pm to 7 am).

All the studied group received and completed a questionnaire concerning age , sex , residence , duration of employments , number of shifts per week, any abdominal pain , alteration of bowel habits especially constipation and diarrhea and symptoms suggestive of peptic ulcer. In addition past history of hypertension was taken.

Finally, the questionnaire covered any psychological, behavioral or mood disturbance with special reference to sleep length, sleep quality , sleepiness at work and morning-time .

Full detailed clinical examination was performed for all subjects.

The control group: Apparently healthy workers were included, comparable to the studied group as regards age, sex, and socioeconomic standard. Members of the control group did not have past history of cardiac troubles, hypertension or diabetes mellitus.

**Laboratory investigations included:**

Serum cortisol ( normally = 5-20 ug/dl ) was measured by competitive enzyme linked immunoassay for quantitative determination of cortisol in human serum.

Serum melatonin MT was determined by enzeme immunoassay for its quantitative determination, apparatus supplied by IBL , Humburg , Germany.

Serum prolactin PRL was determined by radio immuroassay technique (normal level = 1.2 – 29.93 ng/ml).

Serum IgA was determined by turby timer (Nephrymetry) (normal level = 70-400 mg/dl).

Carcino-embryonic antigen (CEA): Serum was stored at -20°C until assayed for CEA. CEA was determined by enzyme immuno assay. For CEA marker, a cut-off value was selected below which levels from 98% of normal healthy women are found. This cut-off level will be 2.5 ng/ml. (Abott laboratories. USA).

Clinical examination and investigations were started at 7 a.m till 9 a.m .to minimize diurnal variations. All participants gave informed concents.

**Statistical assessment:**

Data were collected, checked, revised and entered into the computer. Data were analyzed by SPSS statistical package version 17. Excel computer program was used to tabulate the results, and represent there graphically.

Quantitative were expressed as count and percentages. The differences in distribution were tested by using Chi-square-test at p-0.05 level of significance.

The differences between groups were tested by using independent t-test at p<0.05.

Pearson correlation coefficient was calculated to show the power and direction of the linear relationship between the measured quantitative variables at P<0.05, level of significance.

## Results

Permanent night and rotating shift female nurses have been studied.

Matching of both day shift and rotating shift (workers) nurses, concerning age, duration of employment and BMI is shown in table (1). None of the studied subjects was a smoker. Exclusion criteria were: pregnancy, oral contraceptive use, and regular intake of corticosteroid medications, as well as chronic liver disease.

Table (2): shows that the experience of sleep disturbances (insomnia and sleepiness were prevalent at a rate of 76.09% and 32.6% respectively, among rotating shift workers. Insomnia was found to be the most prevalent complaint (76.09%) followed by gastrointestinal disturbances (56.52%) among rotating shift workers.

All of the previous manifestations were significantly higher in rotating shift workers compared to controls. Also, a statistically significantly higher prevalence of the other clinical manifestations was found among rotating shift workers compared to day shift workers (e.g. Decreased work performance, fatigue-inertia and hypertension).

Table (3) and fig. 1, and 2 revealed that prolactin and cortisol levels were statistically significantly higher among rotating shift workers compared to day-shift

workers. On the other hand melatonin and IGA levels were statistically significantly lower in rotating shift workers in contrast to those of day-shift workers. Moreover, our results showed significantly higher CEA levels among rotating shift workers in contrast to day-shift workers.

As demonstrated in table (4), both cortisol and CEA levels showed a statistically significant positive correlation with both of the duration of employment and worker's age. As regards melatonin levels a statistically significant negative correlation with both variables was found.

**Table (1): General characteristics of the studied groups**

| Variable | Day shift workers (n=20) | Rotating shift workers (n=46) | t-value |
|---|---|---|---|
| | Mean±S.D | Mean±S.D | |
| Age (Years) | 30.50±5.64 | 29.26±4.72 | 0.924* |
| Duration of work (Years) | 10.50±1150.39 | 9.09±4.80 | 1.042* |
| BMI | 23.81±0.59 | 23.74±0.62 | 0.977* |

S.D. = Standard Deviation

* Non significant at 0.05  level fo significance

There is no significant difference between groups by using independent t-test at $p<0.05$

**Table (2): Prevalence of clinical manifestations among the studied groups**

| Manifestations | Day shift workers (n=20) | | Rotating shift workers (n=46) | | $X^2$-value | P-value | |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | | |
| **Sleep Disturbances** | | | | | | | |
| **Insomnia** | 5 | 25.00% | 35 | 76.09% | 22.5 | <0.001 | Highly Significant difference |
| **Sleepiness** | 1 | 5.00% | 15 | 32.61% | 27.12 | <0.001 | Highly Significant difference |
| **Decreased work performance** | 6 | 30.00% | 24 | 52.17% | 10.8 | <0.001 | Highly Significant difference |
| **Fatigue-inertia** | 1 | 5.00% | 18 | 39.13% | 15.21 | <0.001 | Highly Significant difference |
| **Hypertension** | 8 | 40.00% | 22 | 47.83% | 6.53 | <0.001 | Highly Significant difference |
| **Gastrointestinal Disturbances** | 5 | 25.00% | 26 | 56.52% | 15.12 | <0.001 | Highly Significant difference |

\* = $X^2$ test revealed significant difference at $p<0.001$

**Table (3): Comparison between Day shift workers and Rotating shift workers with regard to results of different investigations**

| Variable | Day shift workers (n=20) | Rotating shift workers (n=46) | t-value |
|---|---|---|---|
| | Mean±S.D | Mean±S.D | |
| PRL | 5.76±0.69 | 16.06±11.57 | 3.97* |
| Cortisol | 8.10±2.15 | 26.37±1.52 | 6.02* |
| IgA | 310.41±74.63 | 162.80±2.56 | 8.81* |
| Melatonin (MT) | 13.40±0.10 | 10.73±0.57 | 7.20* |
| CEA | 2.75±0.26 | 5.73±0.38 | 9.75* |

S.D. = Standard Deviation

* = There is a highly significant difference between groups by using independent t-test at $p < 0.001$
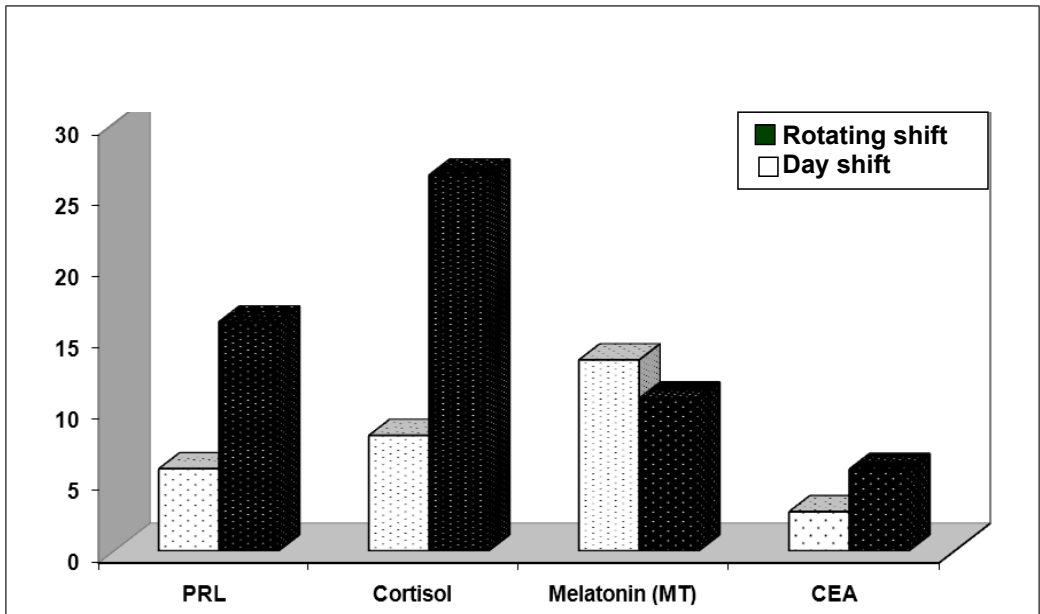
**Fig (1): Comparison between Day shift workers and Rotating shift workers with regard to results of different investigations**

**Table (4): Correlation between different parameters of rotating**

| Variable | Age (Years) | Duration of work (Years) | CEA |
|---|---|---|---|
| Age (Years) | | | 0.709* |
| Duration of work (Years) | 0.993 | | 0.690* |
| Cortisol | 0.788 | 0.781 | 0.571* |
| Melatonin (MT) | -0.609 | -0.649 | -0.510* |

* = Pearson Correlation is significant at the 0.001 level of significant.

## Discussion

Shift work is related to higher risk of sleep disturbances, fatigue and ill health particularly gastrointestinal, metabolic and cardiovascular diseases (Costa, 2003). Shift workers have disturbed sleep pattern and poor sleep quality (De Moss, 2004). In this work, we found that the prevelance of sleep disturbance was significantly higher among rotating shift workers. The same was reported by Wagner (2000), who attributed sleep disturbance and sleepiness among night shift workers to the fact that night work interferes with the production of MT hormone and disturbs its rhythm, a hormone responsible for increasing sleep propensity through thermo-regulatory mechanisms. Moreover, the mood disorders and the decrement of their performance could be attributed to multifactorial causes, however, a major factor is considered to be the mal-adaptation of endogenous circadian rhythm to changes in shift time (Knutsson, 2003).

It was postulated by Metwally (2001) that MT modulates the release of acetylcholine and the pattern of motor activity which might be another explanation of the decreased work performance after night shift. The results of the present study agree with the previous studies and are in accordance with the study of Crowley (2004) who stated that the accumulation of mental and physical fatigue impairs the quality of life of night workers and may be hazardous to their health.

The current study showed a significantly higher incidence of hypertension in rotating shift workers. This is in accordance with Boggild and Knutsson (1999) who concluded that shift workers had 40% excess risk for hypertension and cardiovascular diseases compared to morning shift workers. Also, Cavello et al. (2005) showed that night work stresses were associated with altered cardiovascular responses in healthy nurses and doctors.

Analysis of the clinical manifestations of the studied group showed a significantly higher incidence of disturbed bowel habits. This goes in accordance with a study that showed that gastrointestinal disorders tend to be more common in shift workers than in day workers (Krutsson, 2003). The common complaints were pain and alteration in bowel habits and this was associated with night work in another study (Costa, 2003). The author mentioned that digestive troubles at night work caused mismatch between the endogenous circadian timing system and environmental synchroinzers. Cortisol, a reliable indicator of stress, displays pronounced variation across the time of day with high levels in the morning

and low in the evening. Stress may alter the secretion levels and circadian pattern of the hormone. Increased levels of circulating cortisol lead to high incidence of metabolic, gastrointestinal, cardiovascular diseases (Chroussos, 2000). This agrees with what was revealed in the results of our study.

Katia (2008) attributed the significantly increased levels of serum cortisol during night shift, higher fatigue and worse quality of sleep to a reflection of the arousal state accompanying the state of being awake. Moreover, the basal ACTH and cortisol concentrations were found to be markedly elevated after the fifth day of night shift (Leese et al., 1996). The authors concluded that the pituitary-adrenal responses to corticotropin releasing hormone are disturbed, a condition which mimics the pituitary-fatigue syndrome which might be considered an additional explanation of our results.       The same results obtained by Dahlgen et al. (2005).

In another study serum prolectin PRL showed immediate response to shifted sleep activity cycle and was affected even before melatonin MT and cortisol (Spiegel et al. 1996). Night shift workers showed a nocturnal prolactin peak and the persistence of this peak in night workers raises the question of its significance. It was reported that both prolactin and cortisol could be considered as useful indices of arousal and sleep –wake (Sallinen et al., 2005).

Immunoglobulin A (IgA) is a biologically end point, it can be quantitated and is more stable with a longer biological half-life, than cortisol and catecholamine (Henningsen et al., 1992) and (Becker et al., 1988).

Blood IgA was used as a biomarker for stress-induced immunologic effects in workers. These data explain our results in which there was  significantly lower IgA levels among rotating shift workers in contrast to day shift workers (table 3). However, El-Safty (1999) and Yang et al. (2002) concluded that salivary IgA had particular appeal as a potential biomarker because it can be obtained noninvasively, easily and frequently in comparison with blood.

It was found that melatonin MT presented lower serum concentrations among rotating shift workers in the early morning (at 7 am) after night shift while prolactin and cortisol showed higher concentrations in comparison to those of day-shift workers (Toultou et al., 1990). Our results agree well with the previous results and those reported by Weibel et al. (1999).

As regards the impact of duration of work and the worker's age on MT, PRL

and cortisol blood levels, a significant negative correlation for (MT) and positive correlation for (cortisol) were found with both variables (table 4). This is in accordance with the study done by Weibel et al. (1999) who confirmed that the more the years of shift work, the lower the MT and the higher PRL and cortisol serum levels.

In contrast to our work, Hennig et al. (1998) and Harma and Mikko (1993) concluded that plasma cortisol tended to be suppressed with increasing years of night shift i.e. individual differences in tolerance to shift work were developed.

The suprachiasmatic nucleus in the hypothalamus, one of the most important physiologic determinants of alertness and performance, drives a circadian pacemaker in mammals, with an intrinsic period averaging 24 hours. Light is the primary stimulus to disrupt and reset this pacemaker, which is expressed in changing melatonin rhythm. Light exposure at night may, therefore, be related to a variety of behavioral changes and increased prevalence of fatigue and sleep disorders (Kawachi et al., 1995).

Melatonin MT was found to act as an immune system modulator and as antioxidant to provide substantial protection against free radicles (Reiter and Maestroni, 1999).

Melatonin serum levels in humans decrease when people are exposed to light at night. Suppressed serum melatonin level might enhance tumor development and growth of breast cancer.

Lerchl et al. (1998) suggested that either the pineal glands were directly affected i.e. by an increased calcium influx into pineal photoreceptors, or that the responses were indirectly produced. Moreover, a tumor-promoting effect of light exposure was demonstrated on chemically induced tumors in rodents (Van Den Heligenberg et al., 1999). Melatonin has been shown to be oncostatic for a variety of tumor cells in experimental carcinogenesis (Anisimov et al., 1997). The evidence of a relation between melatonin and oncogenesis in humans is conflicting but the majority of reports indicate protective action of melatonin (Brzezinski, 1997). Several mechanisms have been hypothesized to explain an association between melatonin and the development of hormone dependant cancers as prostate and breast (Schernhammer et al., 2001).

In the present study we measured the carcino-embryonic antigen (CEA) as an indicator of early detection of high risk group of rotating shift workers.

There was a statistically significant difference between rotating shift workers and day-shift workers as regards (CEA) (table 3) which may indicate that (CEA) can be used for early detection or for screening of high risk workers. Several mechanisms have been hypothesized to explain an association between the level of melatonin and cancer.

Melatonin as a new member of an expanding group of regulatory factors that control cell proliferation and loss, is the only known chronobiotic, hormonal regulator of neoplastic cell growth. At physiological circulating concentrations, this indoleamine is cytostatic and inhibits cancer cell proliferation in vitro via specific cell cycle effects. At pharmacological concentrations, melatonin exhibits cytotoxic activity in cancer cells and lowers their invasive and metastatic status. On the other hand, melatonin may induce apoptotic cell death. Biochemical and molecular mechanisms of melatonin's oncostatic action may include regulation of oestrogen receptor expression and transactivation, calcium calmodulin activity, protein kinase C activity, cytoskeletal architecture and function, melatonin receptor-mediated signal transduction cascades and melatonin's circadian stage-dependant tumor growth (Blask et al., 2002).

Melatonin serum levels in humans decrease when people are exposed to light at night. Suppressed serum melatonin levels might enhance tumor development may be via expression of the tumor suppressor gene P53 (Lane, 1994). Evaluation of the relationship between night work, as a surrogate for light exposure at night and breast cancer risk was done by Eva et al. (2001) and they concluded that women who work on rotating night shifts appear to have a moderately increased risk of breast cancer. Also, Stevens (2009) reported the same data.

Both acute and chronic effects of electro-magnetic fields on melatonin production were demonstrated in a study done by El Samra et al. (1999) which demonstrated that reduction of serum melatonin levels was associated with the development of hormone dependent cancers as breast, prostate… etc. The same data were obtained by Zhu et al. (2009) and Forssen et al. (2005).

The results obtained by Heielala et al. (2007) documented that high prolactin levels had close association with breast cancer. Also, Shailendra (2008) found a close association between prolactin and systemic malignancies.

Due to all of the previous researches, we found that, assessment of existing evidence between light-at-night (circadian disruption) and carcinogenesis may be helpful and is better to be studied.

Carcino-embryonic antigen (CEA) is a tumor marker that is overexpressed in many human cancers and functions in vitro as a homotypic intracellular adhesion molecule.

It is a member of cell surface glycoproteins that are produced in excess in human carcinoma at many sites. In general, current clinical applications of CEA may be divided into the categories of detection, diagnosis, prognosis and treatment monitoring (Haagenensen et al., 1978)

In the strictest sense, diagnostic tests are used not in screening, but in case findings i.e. in determining whether disease is present in individuals at high risk for, or suspected of, having the disease (Phil Gold et al., 1997).

CEA has been evaluated in a wide range of malignancies, including breast cancer and historically, has been considered the standard to which new serum markers are compared (Fiorella et al., 1999). These data typically supported the choice of CEA in the present study as a test of monitoring high risk group diagnostic test.

Though not satisfactory, CEA has been used to monitor high risk group (Bigbee and Herberman, 2003). These data support our results in which there is a significant difference between rotating shift workers and day time workers regarding the level of CEA (table 3). However, the level of CEA is still within the normal range in both groups.

## Conclusion

Serum prolactin and cortisol have an immediate response to sleep disturbance and can be used as biomarkers of health troubles of rotating shift workers.

IgA can be used as a biomarker of stress induced immunologic effects in rotating shift workers.

Suppressed serum melatonin level may enhance tumor development. CEA can be used as a tumor marker for high risk group. If the level of CEA is higher than normal, the use of more specific markers is better e.g. CA 125, CA 19-9 and / or CA 15.3.

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