A STUDY OF PATTERN OF SECRETION OF MELATONIN IN SALIVA AND SLEEP PARAMETERS IN SHIFT WORKERS

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APRIL 2015

CERTIFICATE

This is to certify that the dissertation entitled "**Study of pattern of secretion of Melatonin in saliva and sleep parameters in shift workers"** by the candidate Dr.N.S.SASIKUMAR,for M.D Physiology is a bonafide record of the research done by her during the period of study (2012 –2015) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai –600003.

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ABBREVIATION

S.NO	ABBREVIATION	EXPANSION
1.	AASM	American Academy of Sleep Medicine
2.	AHI	Apnea-Hypopnoea Index
3.	BMI	Body mass Index
4.	CSA	Central sleep Apnea
5.	DI	Desaturation Index
6.	ECG	Electrocardiogram
7.	EEG	Electroencephalogram
8.	EMG	Electromyogram
9.	EOG	Electro-oculogram
10.	LSAT	Lowest saturation of oxygen in blood
11.	NREM	Non rapid eye movement
12.	REM	Rapid Eye Movement
13.	PSG	Polysomnography
14.	R&K Criteria	Rechtschaffen and A.Kales
15.	RDI	Respiratory disturbance Index
16.	REM	Rapid eye movement
17.	RERA	Respiratory effort related Arousal
18.	SDB	Sleep disordered breathing
19.	SWS	Slow wave sleep
20.	TRT	Total recording time
21.	TST	Total sleep time
22.	WASO	Wake after sleep onset
23.	PRC	Phase Response Curve

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Study of pattern of secretion of Melatonin in saliva and sleep parameters in shift workers

Introduction

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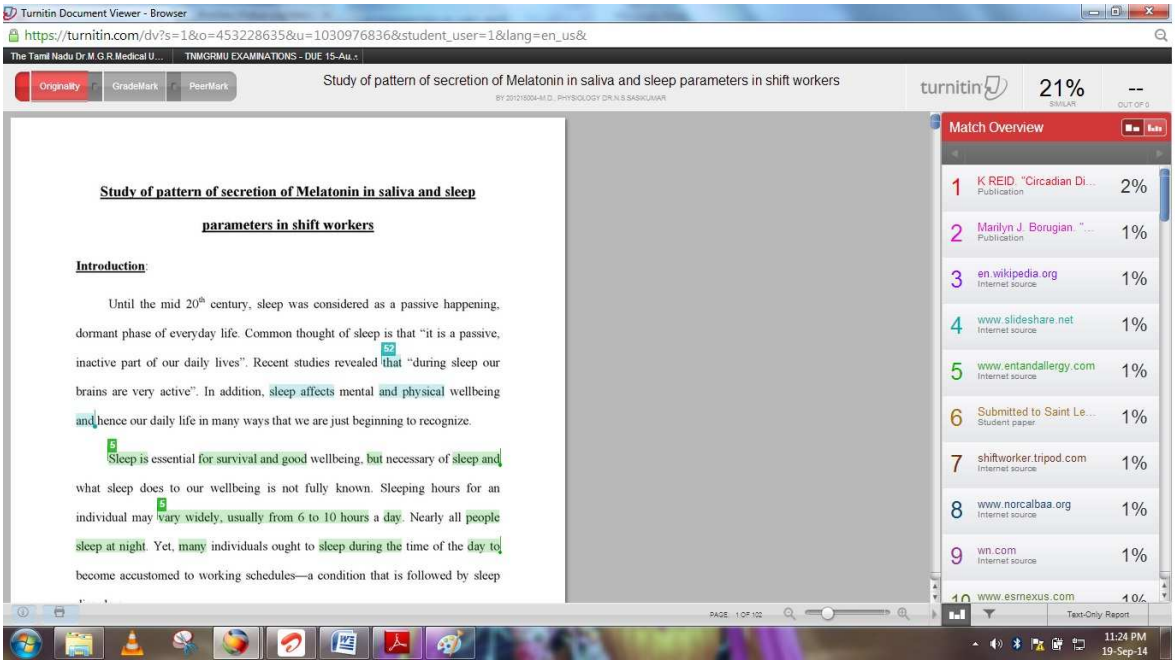
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Introduction:
Until the mid 20th century, sleep was considered as a passive happening, dormant phase of everyday life. Common thought of sleep is that "it is a passive, inactive part of our daily lives". Recent studies revealed that "during sleep our brains are very active". In addition, sleep affects mental and physical wellbeing and hence our daily life in many ways that we are just beginning to recognize.
Sleep is essential for survival and good wellbeing, but necessary 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. Yet, many individuals ought to sleep during the time of the day to become accustomed to working schedules—a condition that is followed by sleep
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ABSTRACT

A STUDY OF PATTERN OF SECRETION OF MELATONIN IN SALIVA AND SLEEP PARAMETERS IN SHIFT WORKERS

Degree for which submitted	:	Doctor of Medicine(MD) in Physiology
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Year	:	2013 – 2014

Back ground:

Globalization and industrialization has lead the shift workers vulnerable for circadian rhythm sleep disorders

Aim & Objective:

To know how far the sleep parameters secretory pattern of melatonin levels in saliva are deviated in shift workers from that of the day workers

Materials &Method:

The study included 30 age matched male shift workers (mean [SD] age: 21.33 [0.48] years) and 30 healthy day workers (mean [SD] age: 24.97 [2.55] years). Polysomnography was performed along with self rated subjective sleep scoring questionnaires. Three Saliva samples for the measurement of melatonin were collected from participants resting in dim light at 2 hr min intervals between 20:00 and 00:00 hr.

Result

Total sleep time, Sleep Efficiency, and duration of rapid-eye-movement sleep showed a significant reduction while Slow-wave sleep (stages 3+4) doesn't show a significant reduction in shift workers as compared with day workers. A significant decrease in time line trend of salivary secretion of melatonin was observed in shift workers. Subjective sleep scores were significantly more towards inadequate sleep for shift workers.

Conclusion:

The circadian phase is altered in shift workers as compared to day workers proved using salivary melatonin concentration as a marker of the same. This study showed shift workers are more prone for sleep disorders, affecting one's health and productivity in society. Early diagnosis, behavioral and life style modification, health education and medication, may alleviate such problems.

Keywords: shift work, polysomnography, melatonin, circadian cycle

STUDY OF PATTERN OF SECRETION OF MELATONIN IN SALIVA AND SLEEP PARAMETERS IN SHIFT WORKERS

Introduction:

Until the mid 20th century, sleep was considered as a passive happening, dormant phase of everyday life. Common thought of sleep is that "it is a passive, inactive part of our daily lives". Recent studies revealed that "during sleep our brains are very active". In addition, sleep affects mental and physical wellbeing and hence our daily life in many ways that we are just beginning to recognize.

Sleep is essential for survival and good wellbeing, but necessary 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. Yet, many individuals ought to sleep during the time of the day to become accustomed to working schedules—a condition that is followed by sleep disorders.

Hours of sleep against age

Children need more sleep per day in order to develop and function properly: up to 18 hours for newborn babies, with a declining rate as a child ages. REM sleep of a newborn is nearly 9 hours a day. After the age

of five REM sleep component declines nearly more than two hours. 10 to 11 hours of sleep is required for school children as evidenced by studies[1].

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

Emotional imbalance or excitement, age, food habits, and use of medications and aging are the factors known to decide the hours of one's sleep and subsequently the feeling of refreshed after waking up. For instance, drugs can cause drowsiness as well as on the other hand make difficult to fall asleep. Caffeine, spicy ingredients, and monosodium glutamate (MSG), may interfere with onset of sleep. Aged people may fall asleep soon as well as they have least adjusting nature to the change of sleeping schedules.

1.1 Physiology of Sleep:

This is widely divided into two following Phases of Sleep according to the multiple electrophysiological recording parameters as Rapid Eye Movement (REM) and Non-Rapid Eye Movement (NREM)

1.2 Stages of Sleep Cycles:

The EEG pattern recorded during sleep varies in a cyclic fashion, which repeats in about every 90 minutes. There are about four cycles in normal 6 to 8 hours of sleep. In normal individuals, sleep cycle begins with slow-wave sleep or Non-REM sleep. There are four stages of slow-wave sleep: stages 1 to 4.

A person when falls asleep, passes sequentially through these four stages of increasingly deep sleep. After that, the sleep lightens and he enters into REM period. With completion of REM phase, sleep cycle completes. The REM phase is followed by the next new cycle, i.e. with stage 1 of non-REM sleep.

Thus, the cycle repeats in every 70 to 90 minutes. Throughout the night, people wake up briefly (called stage W) but are typically unaware of being awake. There are differences in the proportion of time spent in the various sleep stages in different age groups. Moreover, each

individual has his or her own characteristic pattern. Usually, there is a predominance of deep slow wave sleep during the early part of the night, and the first REM sleep may occur after an hour.

REM stage becomes prevalent during the later part of the night. In general, REM sleep occupies about 25 per cent of total sleep period. The duration of REM sleep and stage 4 sleep decrease gradually with advancing age. Newborns and infants sleep about 18 hours a day of which 50% is spent in REM sleep.

1.3 EEG Features of Sleep

In 1953, Aserinsky, Dement and Kleitman through EEG and polygraphic analysis described different phases of normal sleep characterized by EEG, autonomic and endocrine changes.

During wakefulness, EEG usually shows desynchronized, high-frequency, low amplitude known as beta waves in the range of 14 – 30 Hz.

During quiet rest with eyes closed, waves range from 8 to 12 Hz, i.e. alpha waves.

1.4 Architecture

Non-REM sleep:

Stage 1 : Alpha rhythm is replaced by high frequency low amplitude EEG waves.

Stage 2 : Appearance of sleep spindles and K complexes.

Stage 3 & 4 : Delta waves (slow wave)

(Commonly called as deep sleep)

- Delta waves in EEG reflect synchronized oscillations of thalamocortical circuit activity.

REM sleep:

- High frequency, low amplitude activity with PGO spikes

REM is characterized by rapid eye ball movement and profound atonia of other limb muscles (except for extraocular, inner ear and respiratory muscles).

Among these stage 2 sleep occupies50 percent of our total sleep time, REM sleep 20%, and the remaining 30 percent in the other stages.

Infants sleep on the other hand, occupies 50% of their sleep time in REM sleep.

Recent evidences show that “theta oscillations during REM are driven by neurons in the proceruleus area in pons and atonia is caused neurons in the adjacent sublaterodorsal area. (Lu et al, 2006).These ‘REM on’ zones are inhibited by nearby ‘REM off’ area including ventrolateral PAG and lateral pontine tegmentum. ‘REM on’ area can also inhibit ‘REM off’ area. Mutual inhibitions between these areas produce ‘flip-flop switch’ that ensures sharp and complete transition between REM and NREM sleep”.

REM switch is influenced by:

- Cholinergic neurons that promote REM sleep
- Noradrenergic and serotonergic neurons that inhibit REM sleep.

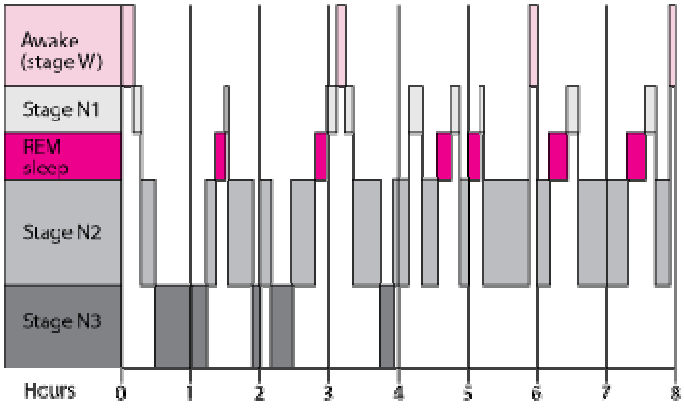


Figure 1 Stages of Sleep Cycle

NREM Sleep-changes in EEG and consciousness (which is primarily a cerebral function) are classified into four stages respective to increasing depth of consciousness. Depth of unconsciousness will become more for as sleep progresses from stage 1 to 4. EEG shows a progressively slower in frequency and higher-voltage pattern (furthermore called slow wave, delta wave)

NREM-Thinking and Body Activities:

Thinking in NREM is short, rudimentary and not able to recollect, Muscle tone is present, and Deep Tendon Reflex can be elicited. Chin and limb muscles exhibit EMG activities.

NREM sleep-autonomic changes:

Widespread decrease in autonomic activities, hypotension and decreased heart rate, and decreased generalized cellular metabolism are noted.

NREM sleep-Hormonal changes:

Growth Hormone, Cortisol and Prolactin secretion occurs mostly in NREM sleep. GH is secreted in 30 to 60 minutes after the beginning of sleep.

Biochemical changes like increased serotonin activity is noted in NREM sleep.

Function of NREM sleep:

Main characteristics of NREM sleep are slow wave in EEG, decreased generalized metabolic activities and deep unconsciousness help revitalize the body

Hence, early night time period is occupied by NREM or slow wave sleep predominantly, while late night phase is occupied by REM which is lighter and dream filled.

EEG/EMG characteristics of REM sleep:

EEG is more active than NREM. Low voltage fast with ocular movement artifact is noted in EEG. EMG is relatively reflecting atonia in REM sleep related to flaccid muscles. While other body activities are as active as like that of awake state.

LATENCIES:

“Sleep latency is defined as the interval to fall asleep after retiring. Normal range is 10-20 minutes”

“REM latency is defined as once asleep, the interval from falling asleep to the first REM sleep is called REM latency”. It may last for 90-120 minutes within one cycle. Variations of above two latencies are useful in diagnosing many sleep disorders. Some disorders may affect one of the two latency and they are distinct to each other.

1.5 Hormone of sleep - Melatonin

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 form contribution of circadian cycle. Its biological activities include causing drowsiness. But the daily cycle is controlled by central nervous system by means of the endocrine and paracrine systems considerably than the melatonin signal alone.

Melatonin (5-methoxy-N-acetyltryptamine,)

Melatonin is a hormone present in all living beings from algae to humans. Chemically it is N-acetyl-5-methoxytryptamine. It exhibits diurnal variation that varies with the time of the day. Melatonin act on melatonin receptors to exert its organic effects. Melatonin also protects nuclear and mitochondrial DNA thus exhibits its antioxidant property.

Production

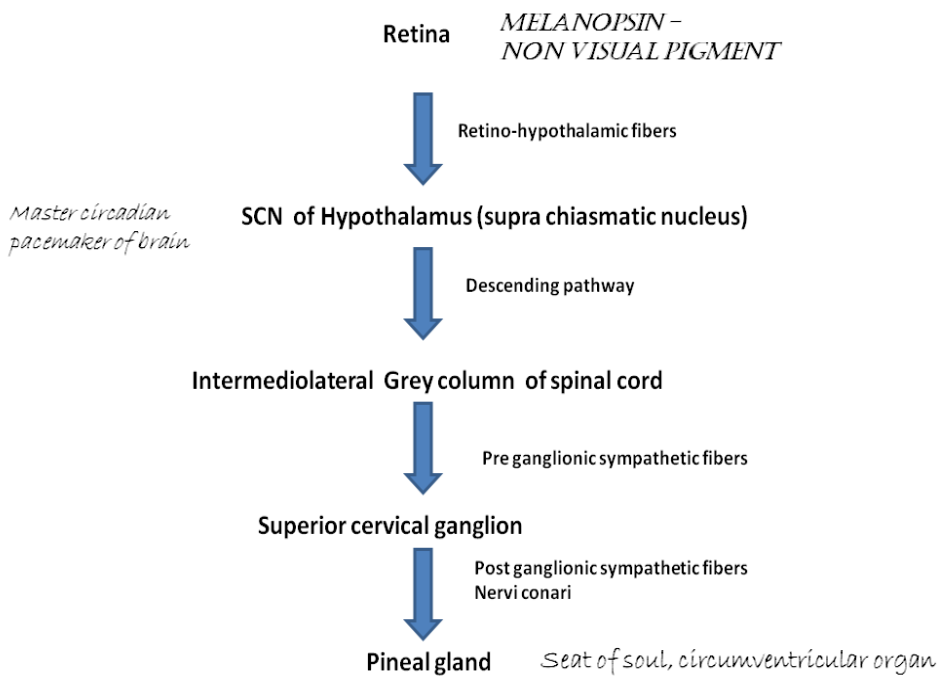
In higher animals, apart from pinealocytes in the pineal gland the retina, lens and GI tract are also tend to produce melatonin. Tryptophan an essential aminoacid forms the precursor for synthesis of melatonin by the enzyme 5-hydroxyindole-O-methyltransferase. Production of melatonin by the pineal gland is under the control of the suprachiasmatic nucleus (SCN) of the hypothalamus. Retina is the one that mediates information about the environmental light pattern daily.

A small group of inner retinal ganglion cells senses the light by special receptors and conveys light/dark information towards SCN. These cells represent 2% of retinal ganglion cells in humans and have the photopigment melanopsin not involved in vision. The sensitivity of melanopsin is with peak sensitivity at 484 nm (blue light). This photoperiod signal entrains the circadian rhythm, thereby subsequent neural and endocrine signals regulates behavioral and physiological circadian rhythms.

Distribution

Melatonin produced and released into the blood like that as an endocrine hormone. While melatonin produced by the retina and the gastrointestinal (GI) tract functions like a paracrine hormone.

Light dependence



Lack of light

↓

incresed adenylate cyclase

↓

increased cAMP

↓ **protein kinase**

phosphorylation of cAMP Response element binding protein

↓

NAT(N Acetyl transferase) Transcription

↓

melatonin synthesis

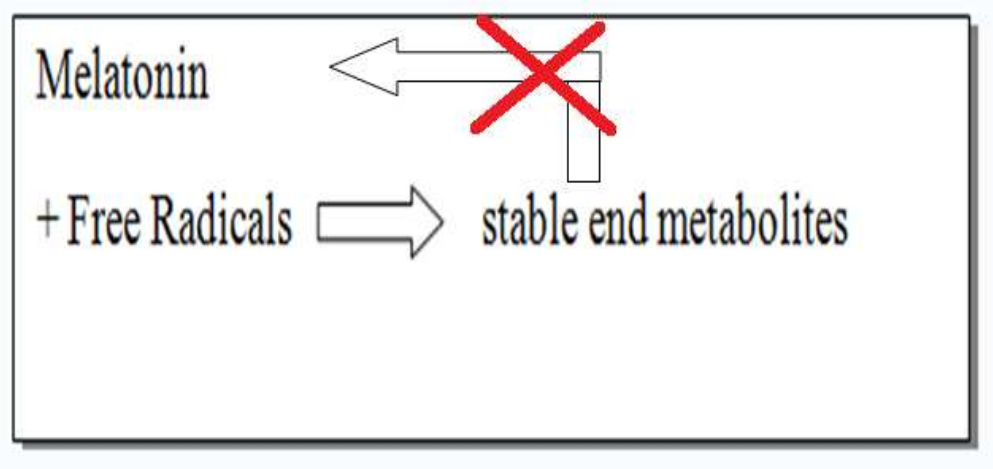
‘Production of melatonin by the pineal gland is inhibited by light and permitted by darkness as shown in above flow chart. It has been called "the hormone of darkness" and its onset each evening is called the Dim-Light Melatonin Onset (DLMO)’. Secretion of melatonin, and its level in the blood, peaks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

Until recent century, humans in temperate zones were exposed to up to 18 hours of darkness in the winter. In the current world, electricity reduces this to on average eight hours or less per day all year round. Low light levels can eb sufficient to inhibit melatonin production to some level. However bright light can reduce melatonin production to greater extent.

“Reduced melatonin production has been proposed as a likely factor in the significantly higher cancer rates in night workers, and the effect of modern lighting practice on endogenous melatonin has been proposed as a contributory factor to the larger overall incidence of some cancers” in the developed world as revealed by some studies.

Antioxidant

Apart from its chief function as synchronizer of the biological circadian clock, anti-oxidant activity of melatonin is also a notable one. In many lower life forms, melatonin functions for only this purpose. 'Melatonin is able to cross cell membranes and the blood-brain barrier'. Contrasting to other antioxidants, redox cycling is not evidenced in melatonin. Redox cycling is the property of a compound to undergo reduction and oxidation repeatedly.



Therefore, it has been referred to as a 'terminal (or suicidal) antioxidant'.

Immune system

While it is clear that melatonin interacts with the immune system, the details of those interactions are unclear. There have been few trials designed to judge the effectiveness of melatonin in disease treatment.

Most existing data are based on small, incomplete, clinical trials. Any positive immunological effect is thought to result from melatonin acting on high affinity receptors (MT1 and MT2) expressed in immunocompetent cells. The increased immune system activity may aggravate autoimmune disorders. In rheumatoid arthritis patients, melatonin production has been found increased when compared to age-matched healthy controls.

Dreaming

Many supplemental melatonin users have reported an increase in the vividness or frequency of dreams. Extremely high doses of melatonin (50mg) dramatically increased REM sleep time and dream activity in both narcoleptics and those without narcolepsy.

Melatonin level

Melatonin level in plasma is found to be in range from 30 to 250 pg/ml according to the decreasing age. Melatonin level progressively declines as the age advances as shown in the table. This hormone also exhibit diurnal variation i.e. its level raises towards the end of the day.

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

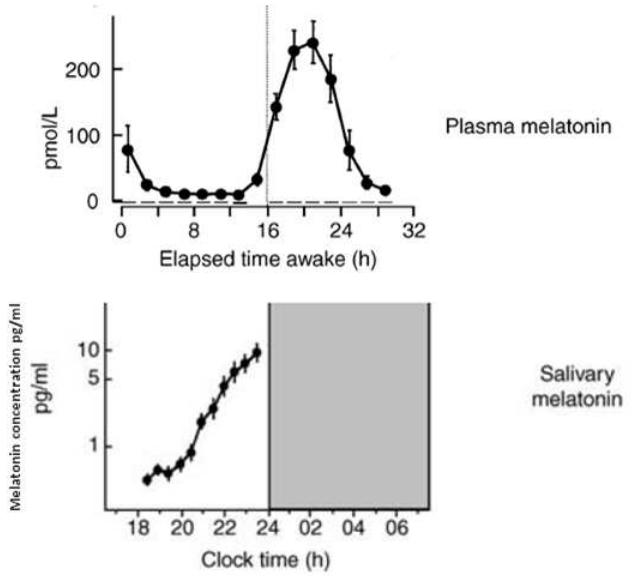


FIG.2 (Above) Time courses of subjective sleepiness as assessed on the Karolinska sleepiness scale plasma melatonin. Adapted with permission from Cajochen et al.[1] (Below) Time courses of subjective sleepiness (for information on the Karolinska sleepiness scale), salivary melatonin in a baseline 7.5-h constant routine followed by a 7.5-h sleep episode. Continuously measured data are plotted in 30-min bins. Adapted with permission from Kra¨uchi et al.[2]

Figure 2 Plasma and Salivary Melatonin versus Time

Light inhibits the production of melatonin by the pineal gland and darkness favors its production. For this purpose melatonin has been called "the hormone of darkness" and its onset during evening is called the

“Dim-Light Melatonin Onset” (DLMO). Dim light favors increased production of hormone. In early hours of the night the hormonal level raises maximum in blood. This is due to increased secretion in initial night hours and gradually falls during the next half of the night (fig: 2). this may vary in timing according to one’s chronotype.

1.6 Physiologic Effects of Sleep:

Nervous system and other functional systems in the body are the two major aspects on which sleep exerts its physiological effects. The effects on nervous system are the more important because any person who has a transected spinal cord in the neck shows no harmful effects in the body beneath the level of transection that can be attributed directly to a sleep wakefulness cycle. Deprivation of sleep has an effect on the normal activity of the central nervous system.

Prolonged wakefulness is usually coupled with progressive malfunction of the thought processes and at times causes abnormal behavioral actions. We are all well-known with the increased sluggishness of thought that occurs after a extended awake period. But as well, a person can become short-tempered or even psychotic after forced wakefulness. Thus, sleep in several ways brings back normal levels of brain activity and normal “balance” among the different functions of the

central nervous system. Since overuse of some brain areas during wakefulness could easily throw these areas out of balance with the remainder of the nervous system. We might postulate that the principal value of sleep is to restore natural balances among the neuronal centers. The specific physiologic functions of sleep remain a mystery, and they are the subject of much research[2] .

1.7 Shift work and Sleep

With the dawn of publicly available electric light in the late part of 19th century, mankind came out of the natural day–night (circadian) cycle. Economic and social requirements are now forcing the construction of a 24-hour society coupled with both economic profit and possible risk of health. A study in the United States, reveals that "more than 15 million people (16.8%) of the full-time workers work on alternate shifts[3](beers TM *et al* 2000)", that is working hours are during periods other than 6 AM to 6 PM. Major health troubles among shift workers include sleep disorders[4] (Akerstedt T *et al 1982*), gastrointestinal disorder, increased incidence of cardiovascular disease, and possible increased incidence of type II diabetes mellitus[5] (Rajaratnam SM,*et al* 2001).

"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 and homeostatic control of sleep. American Academy of Sleep Medicine states that Shift-work sleep disorder consists of insomnia or excessive sleepiness that occurs as transitory phenomena related to work hours that are programmed during the usual sleep period. 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.

Shift work sleep disorder (SWSD) is a type of circadian rhythm sleep disorder in which the endogenous circadian sleep wake cycle is normal and the disturbance arises from controversy between the pattern of sleep and wakefulness generated by the circadian system and the preferred pattern of sleep and wakefulness required by shift work.

Long duration shifts greater than 12 hours and individual susceptibility for phase intolerance may lead to a diagnosis of shift work disorder i.e., those workers on shift time schedule with the greatest sleepiness and performance impairment during the biological night and insomnia during the biological day. Most of the medical disability due to shift work occurs in night or early morning shift workers.

A shift worker trying to sleep in the day time in general sleeps 1.5 to 2 hours less than their night sleeping counterpart. They tend to miss out a major portion of REM sleep and the quantity of stage 2 sleep is not as much of a normal person. This results in "fragmented sleep." Less daytime sleep is a result of the altered sleep/wake cycle as well as concerned with external daytime sound stimuli such as calls, machineries and dogs. It has been revealed that noise stimuli, even if it does not wake one from sleep, has an impression on one's sleep cycles.

The night shift worker while working on nights have got to deal with sleep deprivation in addition to the effect of the trough of the circadian rhythm. Due to this it is difficult for a shift worker to keep from feeling tired.

The objective of this present study is to find the prevalence of Shift Work Sleep Disorder, distribution of their stages of sleep, detectable abnormalities in sleep study and their trough value, amplitude of pattern of melatonin secretion in saliva during onset of night hours, widely known as Dim Light Melatonin Onset in rotational shift workers and comparing the same parameters with age and sex matched day workers.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 .1 Regulation of sleep:

Different factors are involved in regulation of sleep. The main focus has been on the interaction between the homeostatic and the endogenous circadian processes[6](Dijk DJ, *et al* 1995). The homeostatic process accumulates as a function of prior wakefulness, i.e., there is more homeostatic factor the longer you are awake[7](Dijk DJ, *et al*1990). If one is awake for long time, he will get the sleep deeper (increased slow wave activity). The circadian factor on the other hand plays an important role in sleep quantity; that is, sleep duration is for the most part determined by when you go to bed. In other words, sleep length is not dependent on the sleep homeostatic factor, but largely dependent on when you go to sleep according to your own circadian rhythm.

Night workers have experienced this as their sleep duration is usually shorter (often less than 6 h) than normal when going to bed in the morning, even though they often have been awake for more hours before going to bed than daytime workers[8] (Akerstedt T. *et al* 2003). From a practical point of view, this interaction between the homeostatic and circadian processes means that it is important to be awake for a

substantial amount of time to get sleep of high quality, and to have regular bed and rise times in order to have stable sleep duration.

Also habits and behavioral factors have large influences on sleep. We all go to bed at regular hours, not necessarily because we are very sleepy/ tired, but because we know we need to do so, to get enough sleep. Many people experience a high level of sleepiness early in the evening/afternoon, but avoid going to bed knowing that it is not time for bed yet. Behavioral factors can override both the homeostatic and circadian factors. For instance, a night worker is able to stay awake even though both the homeostatic and the circadian factors favor sleep in the middle of the night.

In these instances behavioral factors, like talking to someone, walking around, drinking coffee, increasing illumination, etc., help the night workers to stay awake. Similarly, many teachers have experienced students falling asleep at 9 a.m., even though both the homeostatic and circadian factors favor wakefulness. In this instance, lack of stimulation may be a behavioral factor explaining the increase in sleepiness and risk of falling asleep; that is, a boring lecture, sitting in a dark room, lack of sensory input, etc.

2.1.2 Effect of sleep deprivation

A study done at Research Laboratory “Sports Performance Optimization” National Center of Medicine and Science in Sports (CNMSS), Tunis, Tun isia, evaluated the effect of partial sleep deprivation on the diurnal variations of cognitive performance of handball goalkeepers. 3 cognitive tasks by the use of the reaction time (RT), the stroop, and the barrage tests (to evaluate the RT, the selective and supported attention respectively) following 2 situations of sleep deprivation. The results showed an increased RT and a fall of the level of the attention after the partial sleep deprivation in the afternoon hours. It concluded that partial sleep deprivation affects the diurnal variation of cognitive performance by increasing the RT and reducing the attentional capacities in the afternoon hours[9] (Mohamed Jarraya *et al* 2013).

“A survey of sleep deprivation patterns and their effects on cognitive functions of residents and interns in Korea revealed severe sleep deprivation was associated with higher level of stress, more frequent attention deficit, and difficulty in learning ($P < 0.05$), but not with decreased neuropsychological test results (Hee Jin Kime *et al*)”.

2.1.3 Sleep and memory consolidation:

There is now a large body of literature supporting the notion that sleep plays a major role in memory consolidation. The most prominent theory regarding the mechanism is that recently acquired representations are preferentially reactivated during sleep. In the context of declarative memories, this involves reactivations of memories in the MTLs during slow wave sleep[1011](Peigneux, P., *et al.* 2004 ; Wilson, M. A., & McNaughton, B. L. 1994), which strengthen and stabilize the memories, making them more resistant to interference[12](Diekelmann, S., *et al*2011) and more likely to be retained[1314](Rasch, B., *et al*. 2007; Rudoy, J. D., *et al* 2009).

2.1.4 Sleep - necessary for survival:

Even though we don't know exactly why sleep is needed by people, there are evidences of animal studies that have proved the necessity of sleep for survival. For example, life time of rat is normally two to three years. But the survival time of rat becomes lesser for the REM deprived rats as 5 weeks and as 3 weeks for the rats which are deprived of all stages of sleep. Body temperatures fell abnormally low and sores were noticed on their tail and paws in rats deprived of sleep.

The etiology of sores was found to be compromise in rats' immune system.

Normal functioning of nervous system requires proper sleep. This is obvious in our day to day life, where in sleep deprivation for one day may lead to drowsiness and impaired concentration in memory and deter our physical performance. Hallucinations and mood swings have been reported in prolonged sleep deprivation. Sleep deprivation may lead to break down of neurons due to depletion of energy and accumulation of metabolites of normal cellular activities. Sleep also may give the brain a chance to exercise .Important neuronal connections which are required for prevention of damage from lack of activity and sleep helps in this process.

Deep sleep coincides with the release of growth hormone in children and young adults. Many of the body's cells also show increased production and reduced breakdown of proteins during deep sleep. Since proteins are the building blocks needed for cell growth and for repair of damage from factors like stress and ultraviolet rays, deep sleep may truly be "beauty sleep." Activity in parts of the brain that control emotions, decision-making processes, and social interactions is drastically reduced during deep sleep, suggesting that this type of sleep may help people

maintain optimal emotional and social functioning while they are awake. A study in rats also showed that certain nerve-signaling patterns which the rats generated during the day were repeated during deep sleep. This pattern repetition may help encode memories and improve learning.

2.1.5 Sleep disorders

A sleep disorder is a physical and psychological condition or disturbance of sleep and wakefulness caused by abnormalities that occur during sleep or by abnormalities of specific sleep mechanisms. Although the sleep disorder exists during sleep, recognizable symptoms manifest themselves during the day. Accurate diagnosis requires a polysomnogram, widely known as a "sleep test".

Sleep disorders involve disturbances in the ability to fall asleep, stay asleep, or stay awake or unusual behaviors during sleep, such as sleepwalking. Sleep can be disturbed by many factors, including irregular bedtimes, activities before bed, stress, diet, disorders, and drugs.

The most common symptoms of sleep disorders are insomnia and excessive sleepiness during the day. People with insomnia have difficulty falling and staying asleep and wake up feeling unrefreshed. Lack of sleep makes people feel sleepy, tired, and irritable during the day. People with excessive daytime sleepiness tend to fall asleep during normal waking

hours. Some sleep disorders make people unable to resist falling asleep during the day.

Some sleep disorders involve involuntary movements of the limbs or other unusual behaviors (such as nightmares, night terrors, or sleepwalking) during sleep. Other symptoms may include problems with memory, coordination, and emotions. People may perform less well in school or at their jobs. The risk of having a motor vehicle accident or developing a heart disorder is increased. A detailed description of the problem, sometimes with information from a sleep log, usually indicates the diagnosis, but sometimes testing in a sleep laboratory is needed". This testing includes polysomnography.

2.2 Shift work:

The term "shift work" refers to regular employment outside of the normal "day work" hours. "Human beings are diurnal creatures for whom it is biologically unnatural to work at night, thus leading to the potential for impairments in work alertness and interference with day time sleep. Society expects evenings and weekends to be free for social, religious and recreational activities, thus placing shift workers at a disadvantage, when they are required to be at work during evenings, overnight, or on weekends.

Approximately one fifth of all employees are engaged in some form of work that requires their presence outside of the “standard” 7am to 6pm working day on a regular basis, and can thus be regarded as “shift workers”. “This figure is expected to rise to rise as second jobbing and mandatory overtime increase [15](U.S.Congress, office of technology Assessment: Biological Rhythms: Implications for the Worker (OTA-BA-463). Washington, DC, U.S. Government printing office.1991).

The fastest –growing sector of most western economies is the service sector, and people are increasingly demanding and receiving around the clock availability of such services. This leads to extended works week and fewer different work teams covering each 24-hour day. Physicians are thus increasingly confronted with patients whose conditions may be exacerbated by a failure to cope with the repeated changes in schedule that shift work requires. Some people cope well with shift work, others poorly. At the extreme , moore-ede[16](Moore-Ede MC *et al* 1986) and others have referred to a shift work maladaptation syndrome in those failing to cope. The international classification of sleep disorders [17](ICSD 1990) formally lists shift work sleep disorder as one of the circadian rhythm sleep disorders. The diagnostic and statistical manual of mental disorders [18](American psychiatric association; 1994) lists shift work type as a subtype of circadian rhythm sleep disorder.

Although listed within circadian rhythm-related sleep disorders, shift work intolerance is a problem that should not be regarded as solely a circadian rhythm (biological clock) issue, or asleep disorder issue, or a social and domestic issue[19](Knauth P, *et al* 2003). Rather, it is a complex interaction of these three factors, with each factor influencing both of the other factors and the final outcome of shift work tolerance [20] (Monk TH: *et al* 1998).

	Factors associated with an individual that are likely to cause problems coping with shift work
1	History of gastrointestinal complaints
2	Age>50years
3	working second job for pay (moonlighting)
4	Heavy domestic workload
5	Morning type orientation(lark)
6	History of sleep disorders
7	Psychiatric illness
8	History of alcohol or drug abuse
9	Epilepsy
10	Diabetes
11	Heart disease

	Factors associated with Work systems and Work that are likely to cause problems coping with shift work
1.	More than five third shifts in a row without off time days
2.	More than four 12 hour night shifts in a row
3.	First shift starting time earlier than 7 am
4.	Rotating hours that change once per week(weekly rotation)
5.	Less than 48 hours of time off after a run of third shift work
6.	Excessive regular overtime
7.	Backward rotating hours (1 st to 3rd to 2nd shift)
8.	12 hours shift involving critical monitoring task
9.	12 hours shift involving a heavy physical workload
10.	Excessive weekend working
11.	Long commuting times
12.	Split shifts with inappropriate shift breaks
13.	12 hours shifts with exposure to harmful agents & substances
14.	Overly complicated schedules, which make it difficult to track or plan ahead

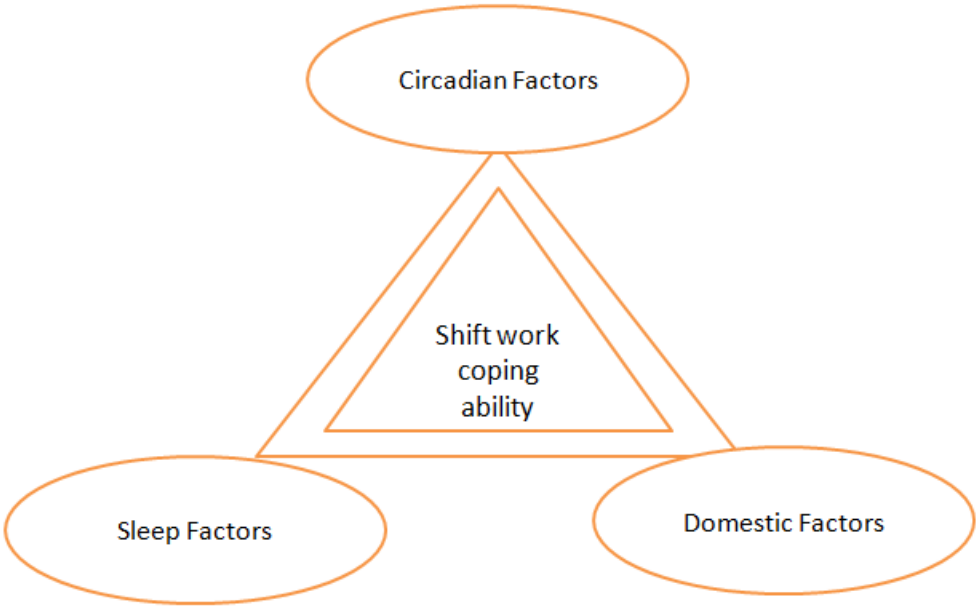


Figure 3 Circadian Rhythm coping ability- Triple Factors

2.2.1 CIRCADIAN FACTORS

"Unfortunately, it is quite clear that, like it or not, HOMO SAPIENS are a diurnal species, biologically hard-wired to be active during the day and sleepy at night. Working at night must therefore be regarded as an inherently unnatural act.

The prime negative influence of the circadian system stems from its inability to adjust instantaneously to the changes in routine that shift work schedules require [2122] (Aschoff J, Hoffmn K, pohl H, *et al*: 1975; Roach GF, Burgess H, Lamond N, *et al*: 2001). Figure illustrates the process of circadian system realignment (as measured by the phase of the

circadian temperature rhythm) in two young volunteers who worked 21 consecutive night shifts[23] (Monk TH, Knauth P, folkard D, *et al*: 1978).

Thus 5 or 6 days were needed before the melatonin onset achieved its desired timing, just prior to the (day) sleep episode. In most work situations, however, this would bring the individual to an off-duty break when a reversion to a diurnal pattern would likely ensue.

As with other investigations, however [24](Czeisler CA, *et al*: 1990), these studies indicated that darkness during the sleep period was an important as the light during the awake period. Thus, complete bedroom light proofing was required, and (more problematically), Eastman and colleagues[25] (Eastman CI *et al* 1994) showed convincingly that dark sunglasses or welder's goggles usually needed to be worn during the morning commute home from night work for the required circadian system phase delay to be accomplished, a procedure that might sometimes compromise traffic safety.

Another way of enhancing circadian adjustment is by taking melatonin pills - a strategy used by many night workers following the attention given to that hormone in the popular press. Whereas there is some laboratory evidence for the effectiveness of melatonin as a chronobiotic[26] (Sack RL, *et al* 1997), its effects are comparatively weak

compared to those of daylight and are likely to be washed out for many shift workers. Slightly different is the concept of using melatonin pills to facilitate daytime sleep (without necessarily changing the timing of the circadian pacemaker). However, although there is good laboratory evidence for such facilitation, double-blind studies of melatonin's effects in actual shift workers have resulted in few definitive improvements in the quality or duration of daytime sleep[27][28](Jorgensen KM *et al* 1998; James M *et al* 1998).

In Europe, many companies are switching to rapidly rotating systems in which only one or two shifts are worked at a time, before a different one is worked[29] (Knauth P, *et al* 1980). Thus, for example, on the continental rotation, employees work two morning shifts, two evening shifts, and two night shifts, followed by two days off. Most European experts favour such systems because they allow the circadian to retain its diurnal orientation, thus eliminating problems of desynchronosis. Because only one or two night shifts are worked before time off is given, sleep loss and fatigue are minimized. The drawbacks of rapid rotation are the circadian-related fatigue experienced during the night shifts".

2.2.2 SLEEP FACTORS

"Sleep is the major preoccupation of most shift workers. In both Europe[30] (Knauth P *et al* 1980) and the United states[31] (Tasto DL *et al* 1978), surveys have indicated that night workers get about 10 hours less sleep per week than their day-working counterparts. Thus, People who happen to need 9 hours of sleep per 24 hours in order to feel well rested very often find shift work extremely difficult to cope with. In his survey of field and laboratory shift work sleep studies, Akerstedt[3233](Akerstedt T *et al* 1985; Akerstedt T *et al* 2005) concluded that the shortening in a night worker's day sleep comes primarily from a reduction in stage 2 and rapid eye movement (REM) sleep, with slow wave sleep relatively unaffected. Not surprisingly, given the prolonged levels of partial sleep deprivation involved, sleep latency can be somewhat reduced in night workers, and some studies have found shorter REM latencies to occur. Essentially, the problem is usually one of sleep-maintenance insomnia rather than sleep onset insomnia.

In this closely protected environment, there was a highly significant difference in duration between the day sleep of night workers and the night sleep of day workers (306 minutes versus 401 minutes, respectively). In addition there were reliable differences between the

polysomnographic characteristics of the sleep, with a smaller amount of REM sleep and a greater proportion of slow wave sleep for the night workers. Thus, even if it were economically feasible, the complete soundproofing and light proofing all shift workers bedrooms would not eradicate the problem of sleep for shift workers.

Unless the shift worker is in a well-adjusted household, his or (more especially) her sleep is liable to be truncated by the demands of childcare, shopping and household management. In viewing the sleep of shift workers, one must therefore consider both endogenous and exogenous factors that are going to limit sleep time.

The use of hypnotics as done in a study of rotating shift workers, Walsh and colleagues[34] (Walsh JK *et al* 1984) found that 0.5mg triazolam could improve the quality and duration of day sleep. However the study was also important in demonstrating that the drug had no significant phase-resetting effects".

2.2.3 Domestic factors

Certainly, if a shift worker's domestic and social life is unsatisfactory, then the individual will not be coping satisfactorily, however well adjusted the sleep and circadian rhythm may be. The additional tasks falls on the female shift worker are child care and

household management. Another aspect of domestic disruption concerns the role of the male shift worker as husband and parent. The factor may increase the risk of divorce by 57%[35] (White L *et al* 1990) in a longitudinal follow up study. Shift worker often suffers from social isolation from day working friends and from religious community organizations.

2.2.4 Coping strategies:

"Employee education programs should emphasize the way circadian, sleep and domestic factors can influence the shift work coping ability. Workers should be taught good sleep hygiene practice and advised how they can manipulate zeitgebers to their advantage, enhancing those that are acting in their favour and attenuating those acting against them. They should also be taught the benefit of prophylactic naps. In some cases family counseling may be indicated to discuss solutions to some of the social and domestic problems. The creation of self help networks can often be of benefit, lessening some of the social and community isolation that many shift workers feel. When educational strategies fail and shift schedule cannot be changed, the patient may require to a day working job.

Management education include convincing the managers first for realizing the potential of problem, and making the wide range of different shift systems that are available, including the rapidly rotating systems so popular in Europe. Recognition of factors such as type of job, nature of workforce, average commuting time, male female ratio, and preponderance of moonlighting that should influence the selection of optimal schedule for that work group in that situation.

Although some people cope well with shift work, many others have significant problems that can adversely affect their health and well being. These problems can become a shift work sleep disorder, which can be quite debilitating to the patient. Shift work problems can be usually understood with a multifactored approach that recognizes the interaction of circadian rhythms, sleep and social and domestic factors in determining shift work coping ability".

2.2.5 Alertness Strategies:

A variety of strategies have been empirically studied and shown to increase alertness in performance. These strategies include planned naps, caffeine, good sleep habits, managing the sleep environment, exercise, light and dark exposure, activity breaks, diet, sedative, hypnotic and

stimulant medication and sleep scheduling[363738](Howard SK *et al* 2002; Rosekind MR *et al* 2002; Rosekind MR *et al* 1999).

Scheduling:

An individual worker can seek every opportunity to increase work hours and income, and an organization or corporation can limit these opportunities in order to reduce potential health and safety risks.

Healthy Sleep:

It is critical that individuals learn about potential sleep disorders that may affect them and seek help from accredited sleep medicine professionals to identify and treat underlying cause of sleepiness.

Conclusion – Managing work schedules:

"Modern work schedules have evolved dramatically from their roots as traditional shift work to meet historical manufacturing and line operations. Effective clinical interventions may involve actions focused on each of these areas for optimal outcomes.

Shift work sleep disorder is otherwise known as circadian rhythm sleep disorder. Its diagnostic criteria are published in the international classification of sleep disorders Revised (ICSD-2).

Delayed sleep phase type (DSPT) and advanced sleep phase type (ASPT) are the two types of circadian rhythm sleep disorder and the former is the most commonly encountered in clinical practice".

Clinical Feature – DSPT:

"It is characterized by sleep onset and wake times that are usually delayed 3 to 6 hours relative to conventional sleep wake times (see figure).

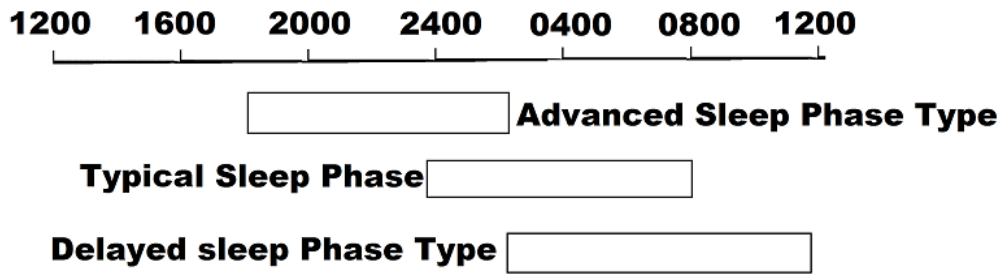


Figure 4 schematic representation of the temporal distribution of sleep and wake in patients with circadian rhythm sleep disorder

Patient typically finds it difficult to initial sleep before sometime between 2 and 6am and when free of social constraints prefers a wake time that is between 10am to 1pm[3940] (Weitzman E *et al* 1979; Weitzman ED *et al* 1981). The clinical picture may be similar to sleep onset insomnia. Enforced conventional wake times may result in chronically insufficient sleep and excessive day time sleepiness. In adolescent the syndrome may be associated with day time irritability and poor school performance

where as in adulthood the symptoms are impaired job performance[41] (Thorpy MJ *et al* 1988) and associated financial difficulty as well as marital problems[42] (Alvarez B *et al 1992*).

Although actual prevalence in general population is unknown it is more common in adolescent and young adults with a reported prevalence of 7%[43](Pelayo R *et al* 1988).In middle aged adults the prevalence may be nearly 0.7%[44](Ando K *et al* 1995). In sleep disorder clinics, 6.7%[45](Weitzman ED *et al* 1981) to 16%[46] (Regestein QR *et al* 1995) patients with primary complaint of insomnia were determined to have DSPT."

2.2.6 Etiopathogenesis:

The tendency for late sleeping is not simply a function of interaction between circadian drive for wakefulness and sleep homeostat but is analogous to other physiological behaviors but overlaid by varying individual emotional social and medical states[47](Regestein QR *et al* 1995). "Late wake times will delay exposure to light in the morning and may prevent active advancement of the circadian clock allowing it to drift to a new phase relation with the external clock time. There is also evidence for genetic basis to DSPT. In some cases it is familiar with autosomal dominant mode of inheritance[4849](Alvarez B *et al* 1992;

Ancoli-israel S *et al* 2001). Polymorphisms in circadian gene such as hPer3, arylalkylamine N-accetyltransferase gene, HLA genes and Clock[50515253] (Archer SN *et al* 2003; Hohjoh H *et al* 2003; Iwase T *et al* 2002; Takahashi Y et al 2000)support the genetic basis".

2.2.7 Diagnosis:

The diagnosis of DSPT is usually made on the basis of the patient's history of chronic or recurrent complaints of symptoms of insomnia resulting from a stable delay in the timing of the major sleep and wake periods[5455](American academy of sleep medicine,2005; American psychiatric association 2000). Sleep log or actigraphy monitoring should be performed for at least 7 days to demonstrate a stable delay in the timing of the habitual sleep period. A morningness-eveningness scale, such as provided by the Horne-Ostberg questionnaire, is also useful to gauge the patients best time of performance. To make the diagnosis, medical, mental or sleep disorders that may cause alterations in the sleep-wake cycle, insomnia or excessive sleepiness should be excluded or adequately treated. Nocturnal polysomnography is sometimes necessary to exclude other sleep-disrupting pathology. When performed during conventional sleep laboratory hours, PSG often shows prolonged sleep onset latency as well as prolonged rapid eye movement sleep latency, and

this many times, in conjunction with an antecedent sleep log, be a clue to the diagnosis.

The use of other physiologic markers of circadian timing, such as a continuous recording of body temperature[56] (Czeisker CA *et al* 1980) or dim light (plasma) melatonin onset (DLMO) [57](Shibui K *et al* 1999), may also aid in determining the phase relationship between circadian and terrestrial time, although routine clinical availability remains limited. DLMO is probably the most useful marker for circadian pacemaker output[5859](Lewy AJ *et al* 1989; Lewy AJ *et al* 1999). In individuals with DSPT, the DLMO times usually occur after 10PM[60] (Shibui K *et al* 1999)(See figure). Determination of DLMO can be made by measurements of melatonin from plasma or saliva. Commercially available salivary determination of DLMO for clinical use may be feasible in near future.

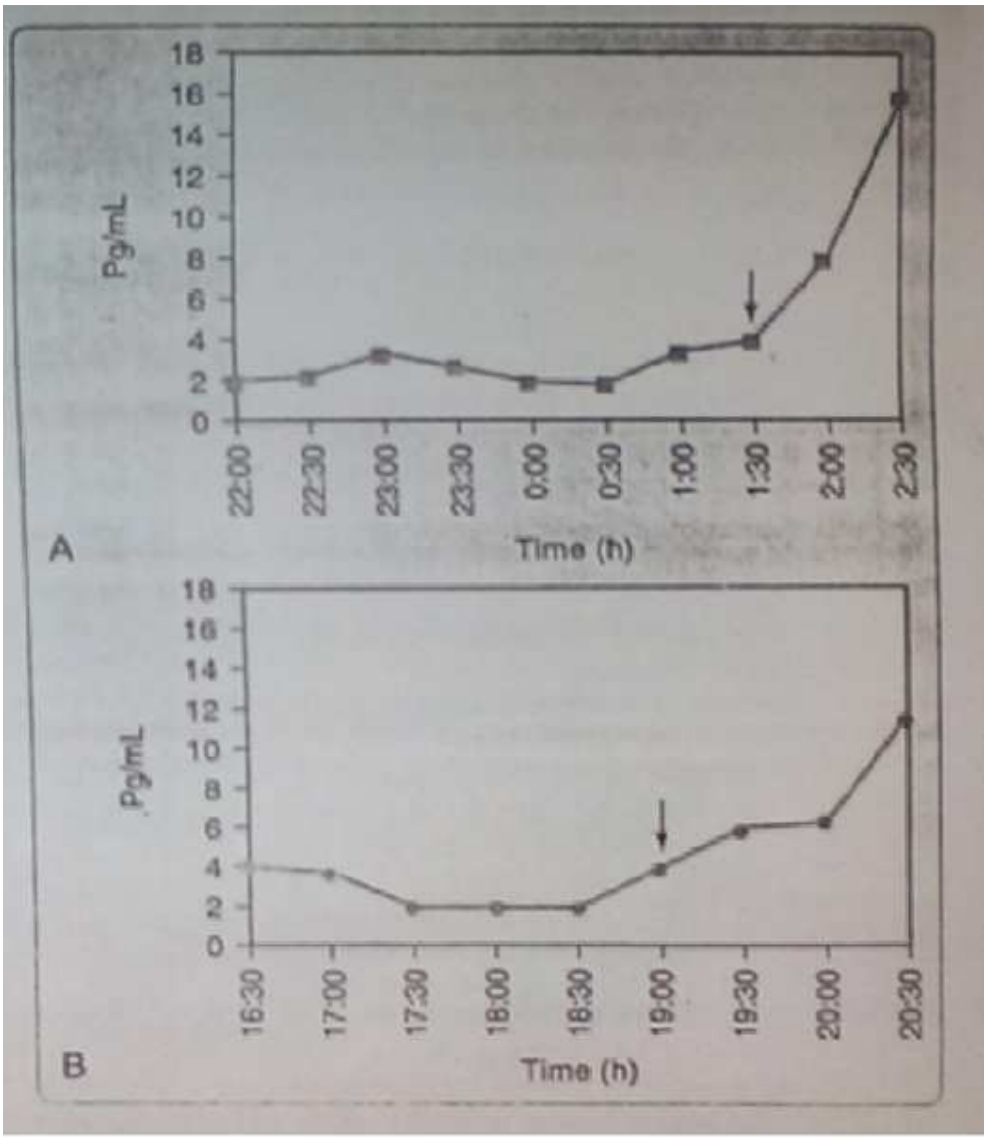


Figure 5 Dim light profiles of individual with Advanced Sleep Phase Type DLMO at 7 pm (top); Delayed Sleep Phase Type with DLMO at 1.30 pm (Bottom) the arrows indicate that the DLMO calculated as two standard deviation above the baselines

2.2.8 Treatment:

The use of chronotheraphy has been successful in small group of patients in laboratory setting[61](Czeisler CA *et al* 1981). Chronotheraphy

requires a successive delay of sleep times by 3 hours daily over a 5 to 6 day period until a desired sleep time is achieved.

This shift is followed by rigid adherence to a set sleep-wake schedule and good sleep hygiene practice. Therapy with bright light in the morning should advance the phase of circadian rhythms in DSPT and may be more practical than chronotherapy[6263](Lewy AJ *et al* 1985;Lewy AJ *et al* 1985). Clinical application of bright light therapy remains empiric and there are no standard criteria for its use in DSPT.

Administration of exogenous melatonin also shifts the phase of endogenous circadian clock. It should be noted that the Phase Response Curve (PRC) for melatonin is nearly opposite to the PRC for light exposure. Melatonin delays circadian rhythms when administered in the morning and advances them when administered in the afternoon or early evening[64](Lewy AJ *et al* 1992s). The relatively small number of clinical studies and variability in dose and time of administration have been limiting factors in the development of a standardized approach for treatment with melatonin. The combination of morning bright light and early evening melatonin may be even more efficacious in creating a phase advance, although clinical data are lacking. In summary the clinical approach to a patient with DSPT should initially include assessment of

circadian sleep phase by sleep diary or actigraphy measures for a period of at least 7 days. Behavioral interventions such as a structured sleep wake schedule, good sleep hygiene practices and avoidance of exposure to bright light in the evening should be prescribed for all patients. In addition exposure to bright light in the morning (1 to 2 hours shortly after awakening) or administration of melatonin in the evening (5 to 6 hours before habitual sleep time) can advance the timing of the sleep wake cycle, although melatonin has not been approved for this indication.

2.2.9 Shift work- a health risk:

Shift work, and night work in particular, is associated with negative effects, such as shortened and disturbed sleep, fatigue, decreased alertness, cognitive decrements, increased injuries and accidents, reproductive problems and risks to cardiovascular and gastrointestinal disease[6566] (Akerstedt T *et al* 1990; Boivin DB *et al* 2007). These symptoms are experienced because shift workers rarely shift their endogenous circadian rhythms to align with the sleep wake schedule demanded by their occupations". Night workers are therefore often in a constant state of circadian misalignment, and both work and sleep at the ''wrong'' circadian phase. The symptoms due to circadian misalignment can be reduced even if the optimal phase relationship is not completely

established. The magnitude of phase shift is positively related to the extent of improved performance and alertness during the night, and better daytime sleep at home(Burgess HJ *et al* 2002)[67].

In 2001, two studies were published suggesting that disruption of the day–night cycle by exposure to light-at-night, especially through nighttime shift work, may be associated with increased incidence of breast cancer (Davis S *et al* 2001; Schernhammer ES *et al* 2004)[6869]. Davis et al[70] reported on a case– control study among women who worked the night or "graveyard" shift, showing a greater risk of breast cancer associated with greater frequency and longer duration of night shift work. Graveyard shift work was associated with an odds ratio of 1.6 (95% confidence interval _ 1.0– 2.5). In the Nurses' Health Study cohort, Schernhammer *et al* [71] examined 2441 incident breast-cancer cases and reported a trend of increasing breast cancer risk with increasing duration on the night shift. They conclude that women who work on rotating night shifts with at least three nights per month have a moderately increased risk of breast cancer after extended periods of rotating shift work. A third study on shift work and risk of breast cancer reported increasing risk by increasing duration of night work (Hansen J *et al* 2001)[72].

"A 2003 study suggested an association between shift work and risk of colorectal cancer (Schernhammer ES *et al* 2003)[73]. Exposure to light at- night may increase risk of cancer by suppressing endogenous production of the neurohormone melatonin (Schernhammer ES *et al* 2004; Stevens RG *et al* 2005; Schernhammer E *et al* 2004)[747576] which has been observed to have potentially beneficial oncostatic, antioxidant, antiadduct, anti estrogenic, and immune modulation activities (Brzezinski A *et al* 1997; Reiter RJ *et al* 2004)[7778]. The antiestrogenic activities of melatonin are highly relevant to breast cancer risk and include interference with the estrogen receptor, reduction of estrogen production, and reduction of circulating estrogen[79](Sanchez-Barcelo EJ *et al* 2005)".

The primary function of melatonin is to convey information on night length to physiologic systems to organize circadian functions such as core body temperature [8081](Claustrat B *et al* 2005; Scheer FA *et al* 2005) as well as seasonal physiology in other mammalian species[82] (Arendt J *et al* 2003). Serum and saliva melatonin levels are normally very low during the day and the levels rise after dark, peaking in the middle of the night. Exposure to light during the dark cycle inhibits melatonin secretion in a dose-dependent manner, with a threshold of 200–400 lux (lumens per square meter) and maximum inhibition after 600 lux for 1 h, 11 as well as being wavelength-sensitive, with maximum effect at

approximately 446–477 nm[8384](Lockley SW *et al* 2003; Brainard GC *et al* 2001).

Future analytic studies of light-at night and cancer would benefit from surrogate measures to estimate lifetime exposure. Occupation, in particular shift work, may be correlated with important patterns of light exposure, but few direct measurements of light exposure in the workplace have yet been published[8586](Koller M *et al* 1994; Dumont M *et al* 2001), and none have reported measurements for periods longer than 56 hours outside the laboratory. Longer measurement periods would allow comparison of rest days with workdays on different shifts or rotation patterns.

The high subjective self rated sleep scores during waking and working hours are consistent with reported fatigue and lack of alertness in shift workers, a major concern in occupational health and safety as with the study done by Borges FN *et al* 2003 and Mistlberger RE *et al* 2003[8788](Borges FN *et al* 2003; Mistlberger RE *et al* 2004).

Measurement of melatonin has been performed primarily in the laboratory as well, using multiple samples of serum, or an overnight urine sample, although one study used salivary melatonin samples (5 per day) 1 or 2 days per week over a period of 4 weeks[89](Lowden A *et al* 2004).

Serum and urine sampling methods have drawbacks, which our three-sample saliva protocol avoided, including the logistics of drawing multiple blood samples, and the lack of information on 24-hour variation available from an overnight urine sample. Research into light-at-night as a cancer risk factor is at a very early stage and measurement is a critical area. This study addressed the feasibility and acceptability of 24-hour exposure measurement for an extended time period to include both workdays and days off in natural residential and occupational settings, and looked at whether years of shift work can be used as a surrogate for exposure to light-at-night.

A study conducted in telecom female workers in New Delhi on various shifts revealed that in case of the rotating shift, the increased demand in the perceptual and motor and the motivational aspects of work caused greater negative influence on physical health symptoms, and social and domestic disruption. The somatic anxiety was greater among women in night work. Findings of the multiple aspects of supported that the behavioral response to the work stressors, and health and well being dimensions did vary with shift schedules. The job design interventions tailoring to the type of work and work place ergonomics might improve the work stressors. The overlapping schedules of the rotating shift, including night work are the documented risk factors. Delayed morning

shift for the operators in the rotating shift and adjustment of shift length based on the work and climatic load (e.g : reduced work hours in the evening shift to avoid peak workload, extend work hours in the day shift during the extremely hot summer months) might alleviate work stresses and enhance health and well being[90](A.Nag *et al* 2004).

2.3.1 Marker of circadian rhythm - Melatonin

For the treatment of Circadian rhythm sleep disorders, an understanding of the circadian timing system is of crucial importance. Sleep usually takes place when the melatonin level is high, and wakefulness normally coexists with low plasma melatonin levels. Based on the close correspondence between sleep/wakefulness and body temperature/melatonin, the core body temperature and melatonin (measured in saliva, urine or plasma) constitute the two most common physiological measures of circadian rhythm[919293](Sack RL *et al* 2007; Brown EN *et al* 1992; Lewy AJ *et al* 1998).

The main site of this endogenous rhythm has been located in the Suprachiasmatic Nuclei situated bilaterally above the optic chiasma in the anterior basal hypothalamus[94](Gillette MU *et al* 1999). Ablation of the Suprachiasmatic Nuclei in mammals has been shown to eliminate circadian rhythms, and transplantation restores the rhythm to the period

of the donor animal[95] (Ralph MR *et al* 1990). There exist several major input fiber systems in the Suprachiasmatic Nuclei. The most important stems from photo receptors in the retina, which covey signals to the suprachiasmatic nucleus via a monosynaptic pathway, the retino hypothalamic tract. Recently it was discovered that the retinal rod and cone cells are not required for photoentraiment, but that there exists a subset of retinal cells (2500 of a total of 100000 cells) containing a light sensing pigment, melanopsin, which is assumed to be involved in circadian photo entrainment[96] (Hattar S *et al* 2002). Measurement of the circadian rhythm based upon the melatonin comprises several samples (normally with a 30 or 60 min interval) of saliva, urine or plasma. The level of illumination currently recommended for sampling is 10 lux[97](Pandi-Perumal SR et al 2007). The most commonly used parameter from these measures is the dim light melatonin onset (DLMO), normally defined as the time when the melatonin level reaches 2pg/ml in plasma [98](Lewy AJ *et al* 1999) or when a level of 4pg/ml is reached in saliva[99](Nagtegaal JE *et al* 1998)".

2.3.2 Treatment with exogenous melatonin

"Exogenously administered melatonin has phase shifting properties, and the effect follows a phase response curve (PRC) that is about 12hour out of phase with the PRC of light [100101102](Sack RL *et al*

2007 Lewy AJ *et al* 1998; Arnedt J *et al* 2005). Melatonin administered in the afternoon or early evening will phase delay the circadian rhythm, whereas melatonin administered in the morning will phase delay the circadian rhythm. The magnitude of phase shifts is time dependent and the maximal phase result when melatonin is scheduled around dusk or drawn [103](Arnedt J *et al* 2005).

The effect of exogenous melatonin is minimal when administered during the night, at least during the first half of the night[104] (Lewy AJ *et al* 1996). Furthermore, similar to the effects of bright light, melatonin administered at an inappropriate time can actually worsen the Patient's condition. Melatonin has in addition to phase shifting properties, soporific effects. This is seen especially when taking melatonin medication during the daytime, when endogenous melatonin is low [105](Arnedt J *et al* 2005). This effect may account for some of its benefit in the treatment of jetlag and shift work disorder.

There is no consensus regarding the appropriate dose or formulation of melatonin. Most studies use fast-release melatonin, but sustained-release preparations are commercially available. The doses used in most studies range from 0.5 to 5mg. Several studies show that the effects of melatonin are not clearly dose-related [106](Sack RL *et al* 2007),

and the phase shifting effect is considered less than those associated with light exposure".

Treatment of circadian rhythm disorders

Exogenous melatonin, usually taken orally in the afternoon or evening, is, together with light therapy upon awakening, the standard treatment for delayed sleep phase syndrome and non-24-hour sleep-wake syndrome.

2.3.3 Studies related to SWSD and melatonin

In a Double-blind placebo-controlled study conducted in Private Suite of a general clinical research center where oral melatonin (0.3 mg or 5.0 mg) was administered 30 minutes prior to each 6.67-hour sleep episode. Both doses of melatonin improved polysomnographically determined sleep efficiency from 77% in the placebo group to 83% for sleep episodes occurring during circadian phases when endogenous melatonin was absent. However, this remained below the average sleep efficiency of 88% observed during sleep episodes scheduled during the circadian night, when endogenous melatonin was present. Melatonin did not significantly affect sleep initiation or core body temperature. Melatonin appeared to maintain efficacy across the study and did not significantly affect percentages of slow-wave sleep or rapid eye movement sleep (Wyatt JK *et al* 2006).

“The study was a randomized, double blind, placebo (PL)-controlled crossover trial, lasting 5 wks. A small dose of melatonin given daily, administered in the afternoon, advanced the sleep timing in teenagers. Twenty-one students, aged 14–19 yrs, with sleep-onset difficulties during school weeks were recruited During the first 6 days in wks 2 and 4, the students received either PL or melatonin (1 mg) capsules between 16:30 and 18:00 h. During the first 6 days of wk 5, all students received melatonin. Wks 1 and 3 were capsule-free. Primary analysis over 5 wks gave significant results for melatonin, sleep and KSS (Karolinska Sleepiness Scale,). Post hoc analysis showed that reported sleep-onset times were advanced after melatonin school weeks compared with PL school weeks ($p < .005$) and that sleep length was longer ($p < .05$). After the last melatonin school week, the students fell asleep 68 min earlier and slept 62 min longer each night compared with the baseline week. Morning melatonin values in saliva diminished compared with PL ($p < .001$) and evening values increased ($p < .001$), indicating a possible sleep phase advance. Compared with PL school weeks, the students reported less wake up ($p < .05$), less school daytime sleepiness ($p < .05$) and increased evening sleepiness ($p < .005$) during melatonin weeks. The concluded that a small dose of melatonin given daily, administered in the afternoon, could advance the sleep timing and make the students more

alert during school days even if they continued their often irregular sleep habits during weekends (Berndt Eckerberg,1 *et al 2012*)".

"A double-blinded study involving One-hundred ninety-two adults (n = 127 women, 65 men; mean age, 46 years) with insomnia on an outpatient basis conducted in department of Neurology Northwestern University Feinberg School of Medicine, Evanston, IL. Both doses of melatonin (0.3 or 3.0 mg) advanced the circadian phase of endogenous melatonin. The magnitude of phase advance in dim-light melatonin onset correlated strongly with the time of melatonin administration, with earlier times being more effective ($r2 = 0.94$, $P < .0001$).These results indicate that melatonin advances the circadian clock and sleep in patients with DSPS in a phase-dependent manner (Mundey K *et al* 2005).

AIMS AND OBJECTIVES

AIM & OBJECTIVE OF THE STUDY

The primary aim of the study is to investigate the pattern of sleep among shift workers and compare their sleep parameters and salivary melatonin levels with the day workers.

Objectives:

1. To assess the polysomnographic parameters during sleep in shift and day workers
2. To assess the salivary melatonin level in shift and day workers
3. To assess the subjective measure of daytime time sleepiness in shift workers using Epworth Sleepiness Scale
4. To assess the subjective measure of quality of sleep using Pittsburgh Sleep Quality Index

MATERIALS AND METHODS

MATERIALS AND METHODS

The study was conducted during the year 2014 at the Institute of Physiology and Experimental Medicine, Madras Medical College after getting approval from Institutional Ethics Committee (IEC), Madras Medical College Chennai.

Selection of subjects

Thirty subjects on rotational shift work with working hours other than 7 am to 7 pm duration of not less than 6 months were selected between age group 20 to 40 years of male gender. Thirty normal day shift workers working on 7 am to 7 pm basis were taken as controls matching age working in offices.

Inclusion Criteria:

Patients both men and women in the age group of 20 – 40 years working on shift work pattern not less than 6 months

Exclusion Criteria:

Patient with the following conditions were excluded from the study:

Obstructive sleep disorder,

Patients with known psychiatric illness, or

Patients on sleep medications,

Pregnancy,

Post-partum period

Subjects with neoplastic, hepatic, diabetic, respiratory and any cardiovascular disorder or other concurrent medical illness (i.e. respiratory and heart failure and renal disease)

Patients with secondary infections

In addition those under medications which affect sleep were also excluded.

Controls were age matched healthy subjects with normal sleep habits.

With these criteria a total of 60 individuals of male gender were selected. Of this 30 were rotational shift workers and remaining 30 were day workers. After thorough explanation of the study, informed verbal and written consent was obtained from the participants, and polysomnography (PSG) was conducted when patient is in stable condition.

STUDY DESIGN: cross sectional study

TYPE OF STUDY: comparative study

PLACE OF STUDY: Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai 03.

POLYSOMNOGRAPHY

STUDY OF NORMAL SLEEP

There are mainly two ways to define the stages of sleep

A. Clinical observations

B. Physiologic information

The term polysomnography was coined by Holland, Dement, and Raynall in 1974 a common method employed to study these changes by recording of Electro encephalogram (EEG), Electro oculogram (EOG), Electromyogram (EMG), Electrocardiogram (EKG), vital signs and breathing parameters. Polysomnography is a comprehensive recording of the biophysiological changes that occurs during the sleep. Polysomnography is usually performed during the night when patient sleeps. Polysomnography is the technique of recording analyzing and interpreting multiple simultaneous physiologic parameters during sleep. Scientific effort was made to characterize all the physiological events that

occur during sleep and provide a standardized scoring manual with universally accepted terminology and specifications.

The manual for the recording and scoring of human sleeping activity was published by A.Rechtschaffen and A.Kales in 1968. Technical advancement, digitization, and discovery of multiple new concepts in sleep medicine, led to incorporation of more comprehensive scoring rules and terminologies by the American Academy of Sleep Medicine (AASM) in 2007. It framed standardized review for specifications, rules for scoring normal sleep and terminology for the evaluation of pathological findings during sleep. AASM uses the term N for NREM sleep stages and R for REM sleep stages, N1 and N2 are used for stage 1 and stage 2; N3 indicates the sum of stage 3 and stage 4. Yet another change is regarding to the nomenclature for recording placements, calling the post auricular placements M1 and M2 (rather than A1 and A2).

The night is divided into epochs time for convenience so that it corresponds to the length of each paper page. The paper speed for speed recording is 10 mm/s ; a 30 –cm page corresponds to 30 s. the segment of time represented by each page is called an epoch. Sleep stage scoring is done in epochs. With technical and digital advancements, sleep recording

at present is done digitally but still sleep is scored conventionally using a 30 second epoch window.

Clinical polysomnogram in addition to these variables is used to diagnose various sleep disorders through airflow monitors of nasal and oral cavity, respiratory effort gauges placed around chest and abdomen, pulse Oximetry – non invasive measure of oxygen saturation, electrocardiogram, EMG of anterior tibialis muscle to detect periodic limb movements and audiovisual means to monitor patient's gross body movements. According to the International Classification of Sleep Disorders (AASM, 2005), there are 90 distinct sleep disorders. Nasal pressure sensors connected to the nose via nasal prongs are accurate in recording apnoea and hypopnoea when compared to thermal sensors (Series F, Mrc I, 1999). During inspiration , airway pressure is negative relative to atmospheric pressure and during expiration , it is relatively positive. The resulting alteration in nasal airway pressure can provide a surrogate estimate of airflow.

Respiratory effort is monitored using respiratory inductance plethysmography which detects changes in inspiratory and expiratory volumes by chest and abdominal wall motion. It also helps to distinguish between central apnoeic event which is not associated with respiratory

effort and obstructive apnoeic event where a phase shift in respiratory effort is recorded. The abdominal wall moves outward due to the descent of the diaphragm while the thoracic wall moves inward due to the negative pressure generated by upper airway collapse.

Transcutaneous pulse Oximetry is used to record different parameters such as total number of desaturations, oxygen desaturation index (ODI), number of desaturation per hour , lowest SpO2, and total time SpO2 spent below 90%. A 4% fall in saturation is considered to be a significant measure of hypopnoea. Oxygen saturation is the oxygen content of blood divided by oxygen capacity and expressed in volume percent. The desaturation recorded is delayed after a respiratory event because of the circulation time from the heart to the finger. The normal delay is about 10 to 12 seconds .

Components of polysomnography recording system include :

- Quality amplifier
- Filter design and configuration
- Independent filter selections for each channel
- Adequate sampling rates and bit resolution for each recorded parameter
- Input signal referencing capabilities

- Provisions for standard calibration procedures and signal verification
- Appropriate signal display

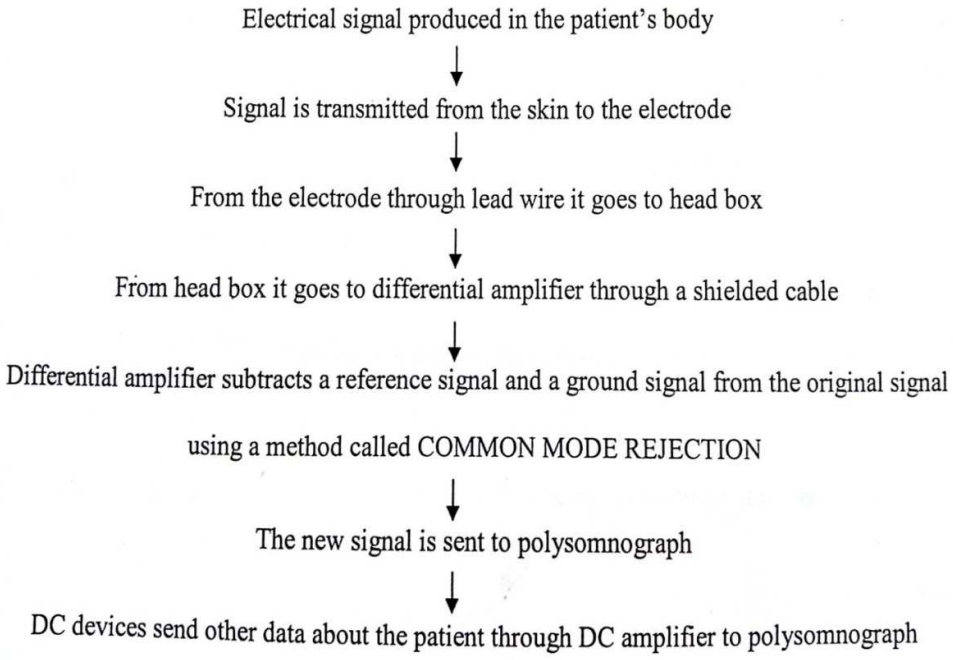
Polysomnography may be digital or analog type. The digital systems are computer based instruments. It has adequate storage capacity . a typical device might require 50 megabytes of storage for an 8 hour recording. Data are usually downloaded onto a computer system separate from the recording device.

SIGNAL SOURCE

There are three sources of signals:

- Bio electric potentials example EEG, EOG, EMG, ECG
- Trasnduced signals from sensors attached to the patient example signal obtained from respiratory, snoring and body position sensors.
- Ancillary equipment signals example Oximeter. These separate instruments have their own processing circuits, signal display and signal outputs.

Signal pathway and Processing in Polygraphic Circuit



There are two types of amplifier – alternating current amplifier to record physiologic parameter of high frequency example EEG, EOG, EMG, ECG and a direct amplifier to record slowly changing potentials as signal from pulse Oximeter.

DIFFERENTIAL AMPLIFIER

It amplifies the difference between electrode inputs rather than the absolute voltage at any electrode.

The electrical noise present at both the electrode is subtracted out and therefore does not contaminate the recording.

The ability of the amplifier to suppress a signal such as noise, that is present simultaneously at both the electrodes is called common mode rejection.

FILTERS:

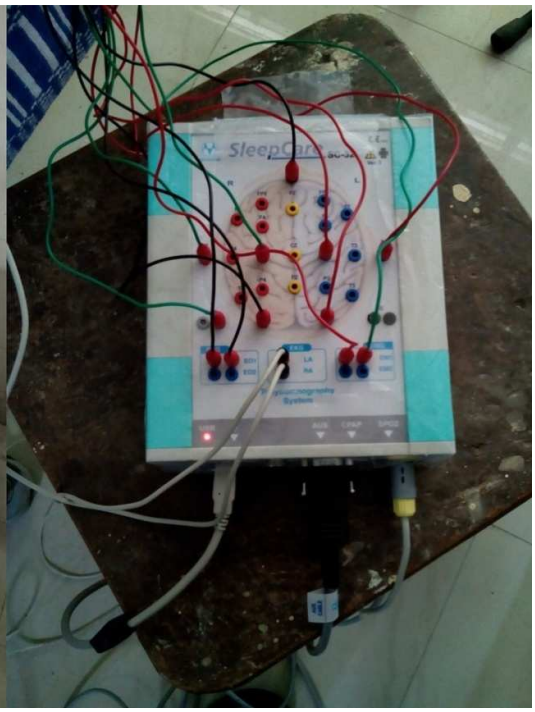
The differential amplifier eliminates most of the unwanted signals and the remaining unwanted signals are removed by the use of filters. Filters are of the following types:

High frequency filter (HFF)- it determinates the upper limit of frequencies that a channel displays at full amplitude . the amplitude of the electrical signal with frequencies greater than HFF settings is attemuated.

Low frequency filter (LFF)- it determinates the lower limit of frequencies that a channel displays at full amplitude. The amplitude of the electrical signal with frequencies lower than LFF settings are attenuated.

Notch filter – 50/ 60 Hz filters are provided as a temporary means of eliminating 50 or 60 Hz frequency interference from amplifier output.

Digital filter - it uses software algorithms to delete selected frequencies after the amplified signals have been converted into digital form.



PATIENT TRAY CONSISTS OF

EEG paste

Measuring tape

Cotton swabs

Electrodes, sensors, and lead wires

Spirit

Micropore

Gloves

Scissors

DIGITAL SPECIFICATION FOR ROUTINE POLYSOMNOGRAPHY (AASM GUIDELINES)

Electrode	Desirable sample rate(Hz)	Minimal sampling rate (Hz)	High frequency filter (Hz)	Low frequency filter (Hz)	Maximum impedance (K Ohms)
EEG	500	200	35	0.3	5
EOG	500	200	35	0.3	5
EMG	500	200	100	10	
EKG	500	200	70	0.3	
Snoring	500	200	100	10	
Airflow	100	25			
Oximetry	25	10			
Chest and abdominal movement	100	25			
Body position	1	1			

SIGNAL MEASUREMENT

The recorded signal can be measured according to

1. Frequency- frequency is described as cycles per second or Hertz which refers to the number of waves appearing in the span of one second.
2. Amplitude – Amplitude refers to the vertical height of a wave and represents electrical voltage of the wave. It depends on the sensitivity setting of amplifier .

Sensitivity is defined as the amount of voltage necessary to produce a set deflection of the pen. Greater the sensitivity, lesser is the recorded amplitude.

REQUIREMENTS FOR CONDUCTING POLYSOMNOGRAPHY

- Air conditioned room with attached bathroom
- Polysomnographic recording system
- Computer
- Amplifiers
- Electrodes and application material
- Pulse oximeter-to detect blood gas analysis
- Abdominal and thoracic belts-to detect respiratory effort
- Nasal airway pressure transducer- to detect nasal airflow
- Access to emergency medical care

Prestudy procedure:

- Detailed sleep wake history is taken
- a complete medical history and clinical examination should be completed
- information is provided to the patient about the purpose and procedure of sleep study
- Patient should be made aware that their sleep will be monitored throughout the entire study and they should be told how to contact the technologist if necessary.

The patient was asked to follow the instructions:

- To take a bath in the evening and shave of facial hair
- Not to apply oil anywhere on the body
- To take dinner at least one hour before sleep study
- Not to consume alcohol on the day of study
- Avoid coffee or tea at least 3 hours before the study
- Dress in routine sleep wear
- Remove all ornaments
- To bring all previous medical reports
- Report for sleep study at the appointed time

SLEEP STUDY PROCEDURE:

Patient hookup: - it involves placement of various sensors to record the different parameters during sleep.

ELECTROENCEPHALOGRAM:

EEG is the surface recordings of the summation of excitatory and inhibitory postsynaptic potentials generated by pyramidal cells in cerebral cortex. The advantage of EEG recordings in research is their non – invasive nature and high temporal resolution so that changes of even a few milliseconds can be detected. Recordings reveal subtle differences in brain function, which are not always accompanied by behavioural performance.

The disadvantage is their low spatial resolution and they only reflect synchronous electrical activity of large neural assemblies. Contamination of EEG recordings by artifacts (eye or head movements, muscle movements, and external electrical noise) compromises the interpretation of the data if such artifacts are not removed. The EEG is recorded using six “exploring” and two “reference” electrodes.

The acceptable derivation for the purpose of scoring sleep stages are C3 or C4 referred to opposite mastoid; two channels are recorded with

one used as backup for technical problems during night. The opposite side derivation is used to maximize inter electrode distance as amplitude of EEG is dependent on inter electrode difference and the criteria for scoring sleep stages III and IV are dependent on amplitude.

International 10 -20 system was developed as a standard measurement tool for placing electrodes on the head for recording EEG. The following electrode sites are located according to R&K criteria – two mastoid (known as aural , A1, A2), two central (C3, C4) and two occipital (O1, O2) . EEG is recorded using either gold cup or silver chloride electrodes. Electrode impedance should ideally be less than 500 ohms. Standard gain for EEG electrodes is a deflection of 1 cm for every 50 µv. Recommended EEG deviations are : C4-A1, O2-A1, with C3-A2 as a backup.

STEPS IN INTERNATIONAL 10-20 ELECTRODE PLACEMENT SYSTEM

Location for four landmarks-nasion, inion, left preauricular region , and right preauricular region.

The distance from nasion to inion was measured. The 10 % of total distance from nasion is marked as Fpz and from inion as Oz along the

line joining them. The halfway point between nasion and inion is marked as Cz. 20 % distance from Cz in front is marked as Fz and back as Pz.

The distance between left and right preauricular point was measure, with the tape passing through the halfway mark of Cz. From the Cz 20 % of the distance on the left is marked as C3 and right as C4 on the line joining the left and right preauricular points.

The circumference of the head is measured by passing the through all the 10% marks. 50% of this measurement coincides with Oz at the back and Fpz in the front. 5% of circumference to the left of Oz is marked as O1and right as O2. Similarly 5% circumference to left of Fpz is marked as Fp1 and right as Fp2.

The distance from Fp1 to O1 passing through C3 is measured. 50%of this distance should intersect at C3. 25% of this distance is marked on the line joining Fp1and C3 as F3 and similarly on the right side as F4.

A1 and A2 are placed behind the ears on the mastoid process.

For recording the electro oculogram, the placement of an electrode is located 1 cm out and below the outer canthus of the left eye (E1) and another electrode placed 1 cm out and above the outer canthus of right

eye (E2) with reference to the right mastoid process (A2). It records the retino- corneal potential difference .

For recording the electromyogram of the chin ,two electrodes are placed just below the chin , one electrode placed 2cm below and 2cm left of midline and the others is 2cm below and 2cm to the right of midline.

Respiratory effort belts are placed snugly around the thorax and abdomen.

Airflow sensors- Nasal prongs connected to a pressure transducer is used for measuring nasal flow (gives an indirect measure of flow based on the recording of the pressure at the gives an indirect measure of flow based on the recording of the pressure at the prongs relative to the atmospheric pressure.)

Oximeter sensor is placed on any of the three middle fingers. It is a noninvasive method for monitoring the hemoglobin percentage saturated with oxygen. Snore sensor is also connected.

Leg EMG leads records activity of anterior tibialis muscle. Electrodes are placed on the outer aspect of lower half of each leg.

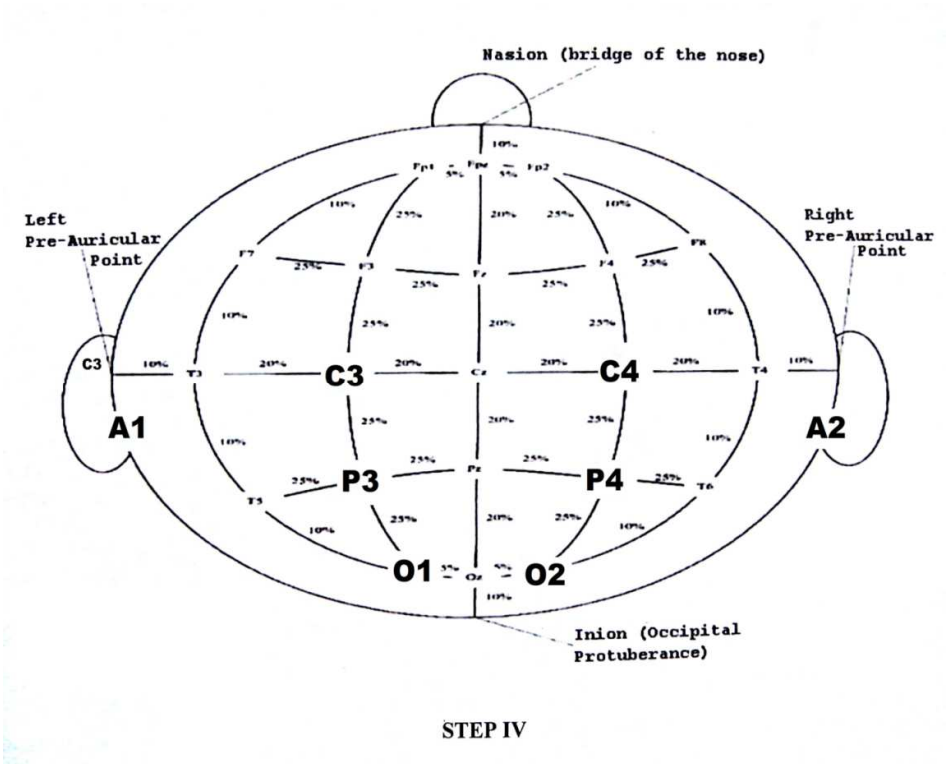


Figure 6 **10 -20 system of electrode placement for EEG**

PARAMETERS RECORDED:

- ELECTROENCEPHALOGRAM
- CHIN ELECTROMYOGRAM
- ELECTRO OCULOGRAM
- ELECTROCARDIOGRAM
- AIRFLOW
- ABDOMINAL AND THORASIC RESPIRATORY EFFORT
- LEG/ARM ELECTROMYOGRAM
- SATURATION

- SNORING
- BODY POSITION
- HEART RATE

After all sensors are applied, the impedance and signal of all the electrodes was checked. Physiologic biocalibration was done at the start of the study before lights out and just after lights on at the end of the study.

Close eyes-instruct patient to lie down with eyes closed for 30 seconds. This helps to reveal the alpha activity.

Open eyes-instruct patient to lie still down with eyes open for 30 seconds. This helps to eliminate the alpha activity.

Look left and right- instruct patient to look to left and right repeatedly while holding the head still. This mimics the eye movement seen during REM sleep

Look up and down- instruct patient to look to the up and down repeatedly while holding the head still. This differentiates the vertical and horizontal eye movements.

Hold breath-Instruct the patient to take a deep breath and hold it for 5-10 seconds. This mimics the central apnoea

Respiratory effort- Instruct he patient to move the chest and abdomen in and out while holding breath. This mimics an obstructive apnoea.

Move feet- Instruct the patient to move the feet. This mimics leg movements during sleep.

During the study the observer should document

Time of sleep study

Biocalibration

Technical difficulties and methods of correction

Patient complaints

Post study procedure

Ensure that study is saved properly

Clean and sterilize the electrodes and various sensors

Scoring and data analysis is done to recognize the following events

Sleep staging

Arousal

Cardiac events

Respiratory events

Movement events

SCORING BY EPOCHS

The polygraph record is divided into segments of equal size, with epoch length of 300mm and duration of 30 seconds.

A single stage score is assigned to each epoch-when more than one stage is present in each epoch-the stage score of the epoch is determined by the stage that takes up the greatest portion of the epoch.

SLEEP STAGES SCORING

Sleep stage are scored at 30 sec sequential recordings known as epochs.

Wake – A>50% of an epoch has alpha EEG waves over the occipital region with eye closure or if alpha waves absent the presence of any of the following

Eye blinks (0.5-2Hz)

Reading eye movement which consists of slow conjugate movement followed by a rapid movement in the opposite direction,

Rapid open eye movements associated with normal or high chin tone.

Stage-I- alpha waves being replaced by a low amplitude , mixed frequency (4 to 7Hz) waves occupying >50% of the epoch, or the presence of (for those who do not generate alpha waves)

Vertex sharp waves of <0.5 seconds duration that are maximal over central region

Slow eye movement

Stage II- defined by the presence of K complexes or sleep spindles.

Stage III and IV – indicated when >=20%of the epoch shows slow wave (0.5-2Hz and >75uV) EEG activity.

REM – presence of low amplitude, mixed frequency EEG activity , rapid eye movement on EOG, and low tone on EMG.

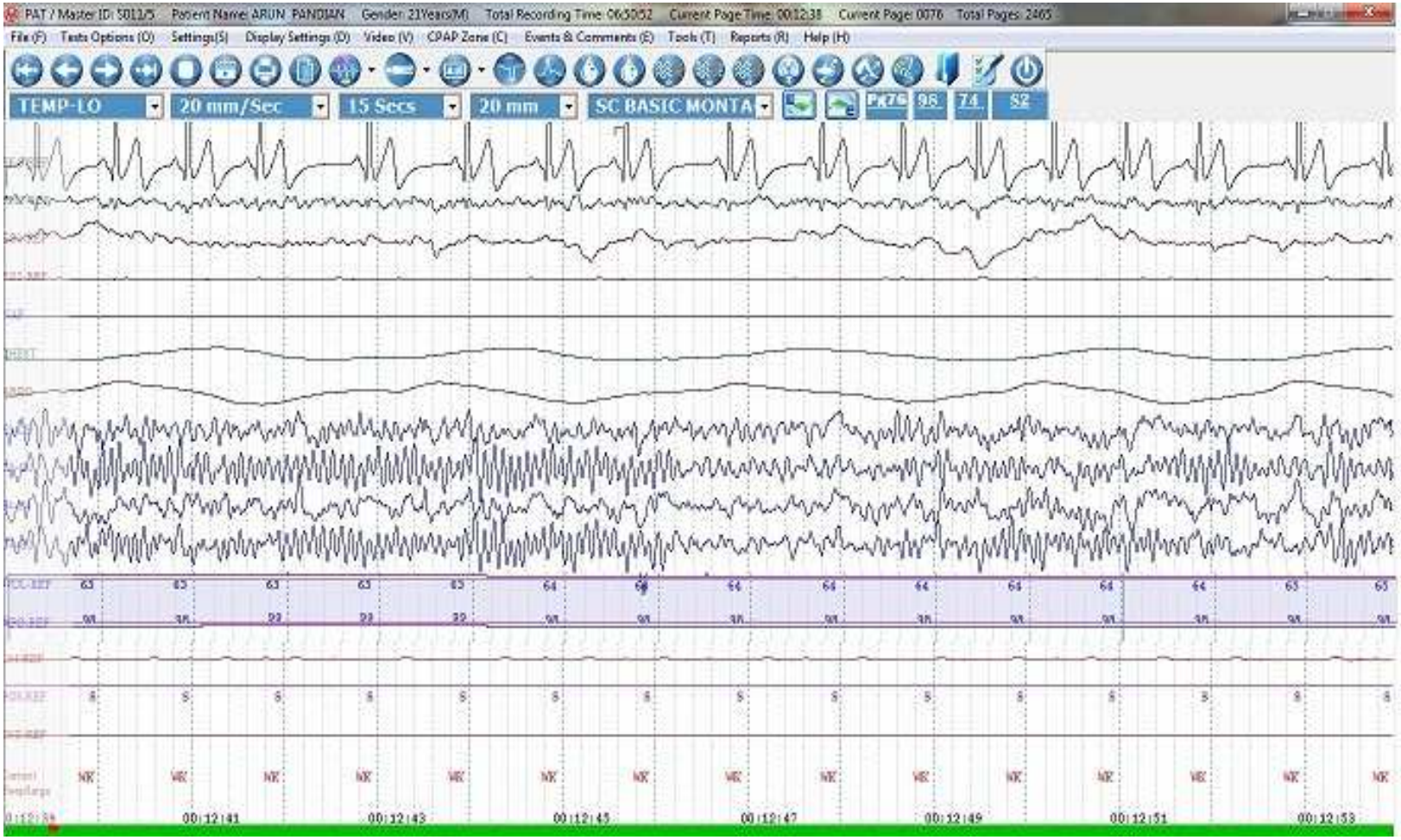


Figure 7 Epoch of awake stage

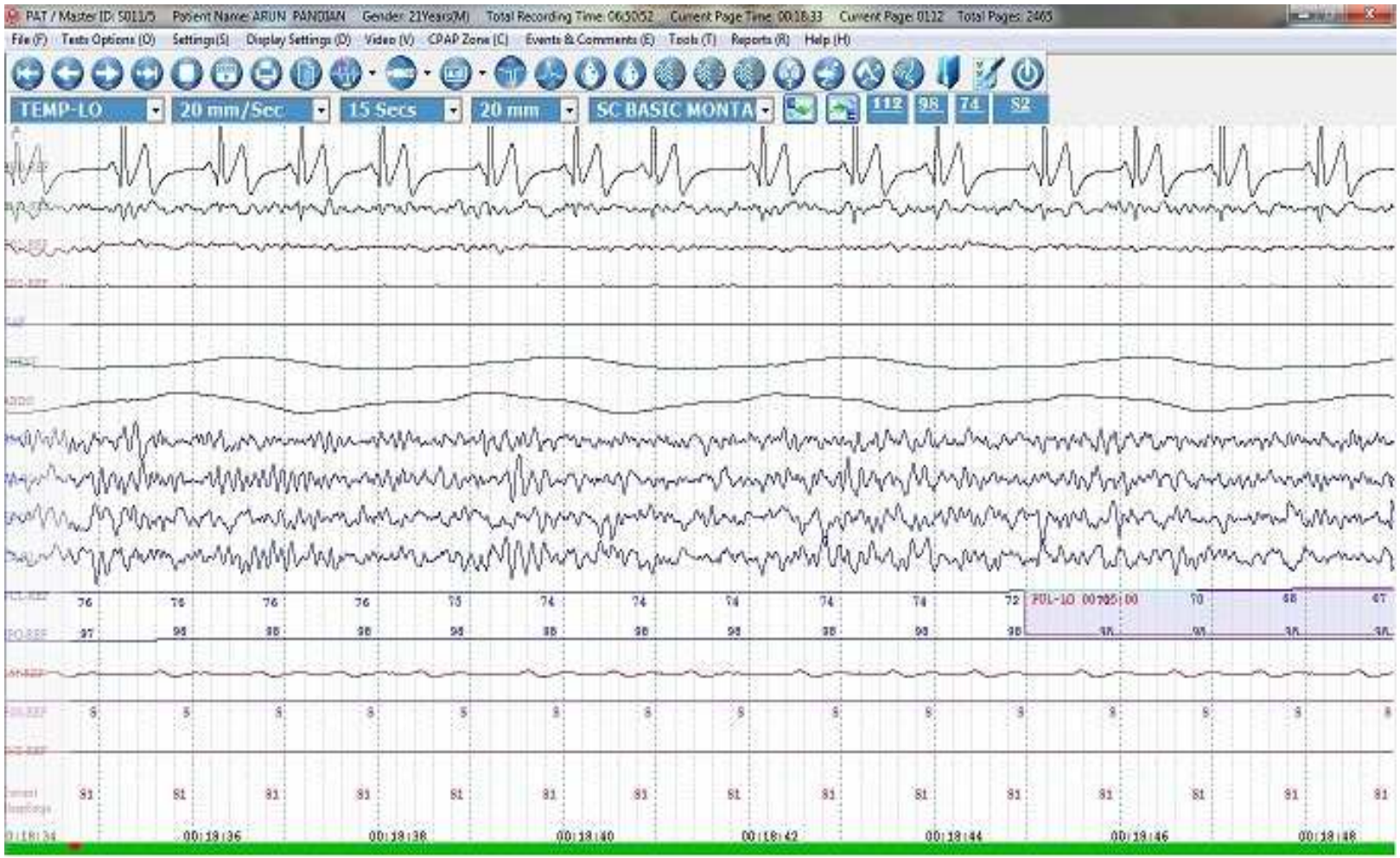


Figure 8 Epoch of stage N1

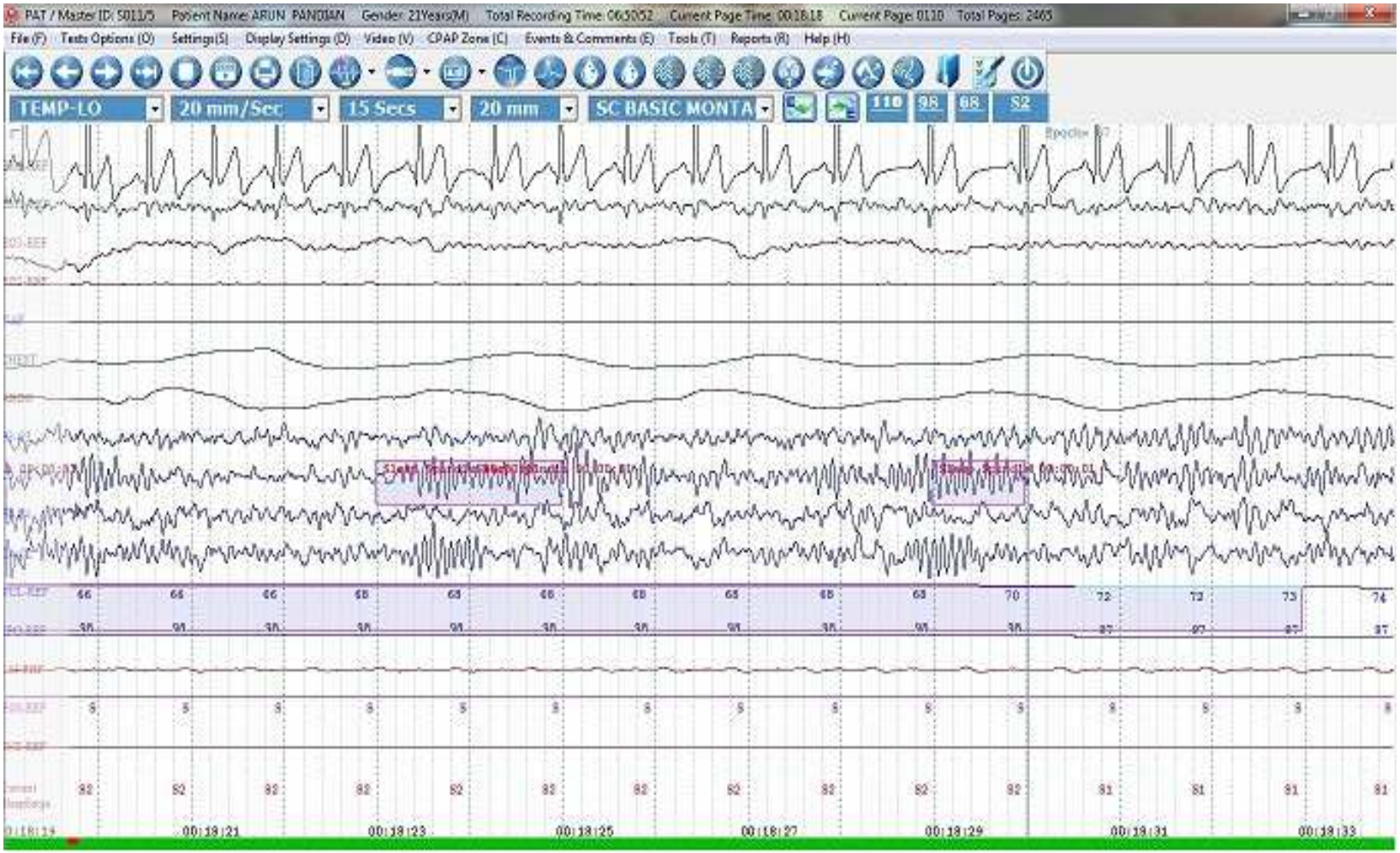


Figure 9 Epoch of stage N2

Figure 10 Epoch of stage N3

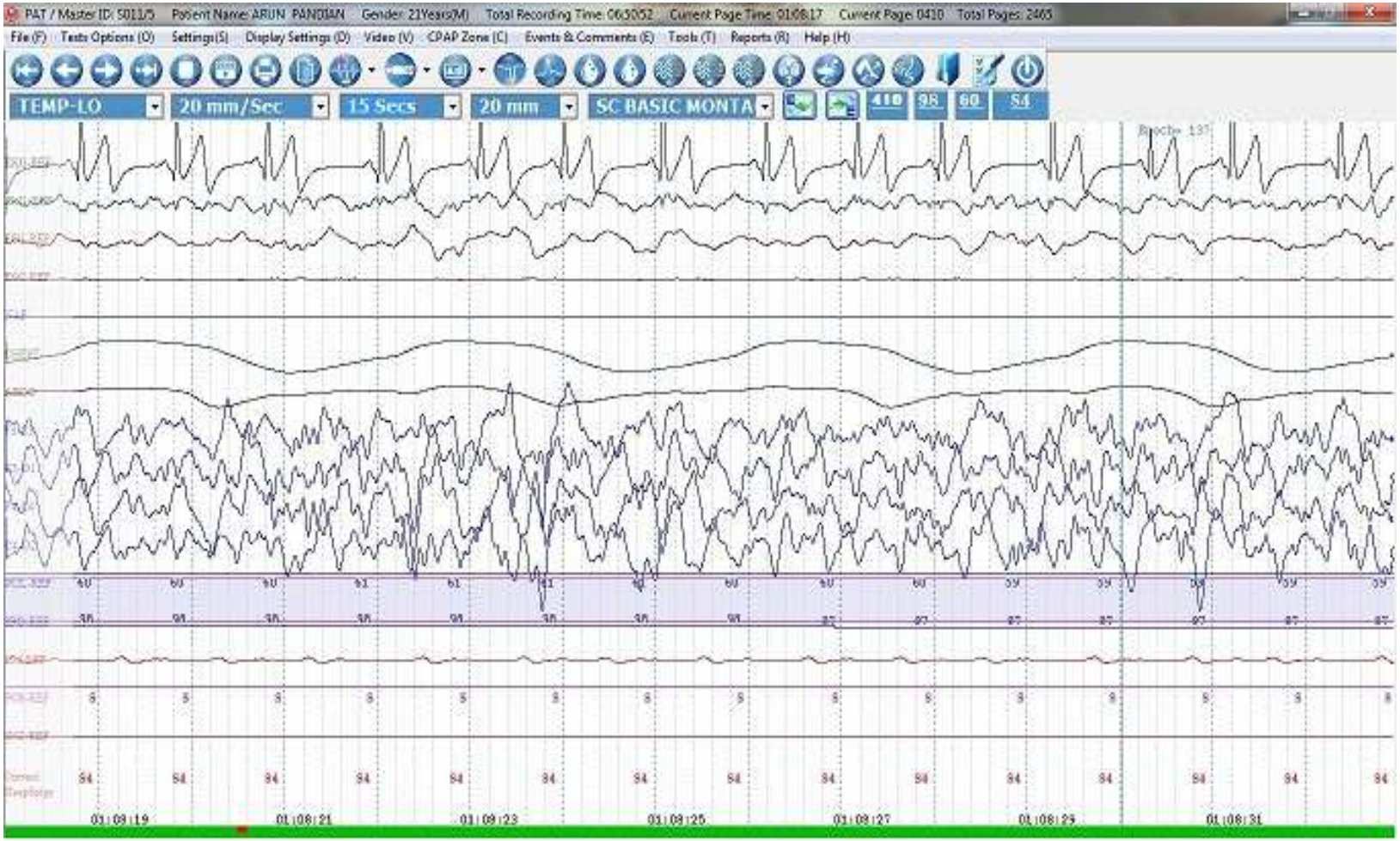


Figure 11 Epoch of stage N4

Figure 12 Epoch of Rapid Eye Movement stage

RESPIRATORY EVENTS SCORING RULES:

APNOEA-decrease in airflow amplitude by =>90% from baseline for a duration of minimum10 seconds. It is scored as

Obstructive-if inspiratory effort is present throughout the entire period

Central -if the inspiratory effort is absent throughout the period

Mixed –if inspiratory effort in the initial part of the period is followed by the presence of inspiratory effort.

Hypoapnoea-decrease in airflow amplitude by =>30% of baseline for duration of at least 10 seconds accompanied by =>4% of oxygen saturation.

Arousal

During NREM sleep – abrupt frequency shift (eg.alpha , theta or frequencies >16 Hz) lasting =>3 seconds and preceded by =>10 seconds of stable sleep.

During REM sleep abrupt EEG frequency shift (e.g alpha, theta, or frequencies >16 Hz) lasting =>3 seconds and preceded by =>10 seconds of stable sleep, accompanied by an increase in chin EMG that is =>1 second duration.

Respiratory Effort Related Arousal (RERA)- breaths associated with heightened respiratory efforts or flat airflow waveform with a duration =>10 seconds and preceding an arousal but not meeting criteria for either apnoea or Hypoapnoea.

Periodic limb movement in sleep

Four consecutive leg movements (each 0.5 to 10 seconds in duration with an amplitude 8uV above resting EMG) characterized by period lengths of between 5 and 90 seconds between onset of consecutive movements.

Leg movements on different legs are counted as one movement if they are separated by <5 seconds between movement onsets.

SLEEP ARCHITECHTURE DEFINITION AND FORMULAE

% Stage I	=	Minutes of Stage I *100/ Total SleepTime (TST)
% Stage II	=	Minutes of Stage II *100/ Total Sleep Time(TST)
% Stage III & IV	=	Minutes of Stage III&IV *100/ Total Sleep Time(TST)
% Stage R	=	Minutes of Stage R *100/ Total Sleep Time(TST)
% Wake time	=	Minutes of Wake *100/ Sleep Period Time (SPT)

Sleep Onset	=	The first three consecutive Epochs of Stage I sleep
Latency to REM	=	The period of time from sleep onset to first Epoch of REM
NAP onset (Sleep) latency	=	the period of time to lights out to sleep onset
Sleep Period Time (SPT)	=	The time from sleep onset to last Epoch of sleep
Total Recording Time (TRT)	=	The time from lights out to lights on
Total Sleep Time (TST)	=	The amount of sleep recorded during TRT
Wakefulness After Sleep Onset (WASO)	=	The Wakefulness occurring from sleep onset to last Epoch of sleep

OTHER INDICES

Apnoea Hypoapnoea Index (AHI)	Total number of apnoeas and Hypoapnoeas occurring per hour of sleep
Respiratory Disturbances Index (RDI)	Total number of apnoeas and Hypoapnoeas and Respiratory Effort Related Arousals occurring per hour of sleep
Desaturation Index	Total number of desaturation events per hour of sleep

4.3SUBJECTIVE SLEEP ASSESSMENT QUESTIONNAIRES

PITTSBURGH SLEEP QUALITY INDEX (PSQI)

INSTRUCTIONS: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, when have you usually gone to bed at night?

   USUAL BED TIME_____

2. During the past month, how long (in minutes) has it usually take you to fall asleep each night?

   NUMBER OF MINUTES_____

3. During the past month, when have you usually gotten up in the morning?

   USUAL GETTING UP TIME_____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spend in bed.)

   HOURS OF SLEEP PER NIGHT_____

INSTRUCTIONS: For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you...

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a) ...cannot get to sleep within 30 minutes	☐	☐	☐	☐
(b) ...wake up in the middle of the night or early morning	☐	☐	☐	☐
(c) ...have to get up to use the bathroom	☐	☐	☐	☐
(d ...cannot breathe comfortably	☐	☐	☐	☐
(e) ...cough or snore loudly	☐	☐	☐	☐
(f) ...feel too cold	☐	☐	☐	☐
(g) ...feel too hot	☐	☐	☐	☐
(h) ...had bad dreams	☐	☐	☐	☐
(i) ...have pain	☐	☐	☐	☐

(j) Other reason(s), please describe

_____

_____

How often during the past month have you had trouble sleeping because of this?	☐	☐	☐	☐

	Very good	Fairly good	Fairly bad	very bad
6. During the past month, how would you rate your sleep quality overall?	☐	☐	☐	☐

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?	☐	☐	☐	☐
8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?	☐	☐	☐	☐

	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	☐	☐	☐	☐

	No bed partner or roommate	Partner/ roommate in other room	Partner in same room, but not same bed	Partner in same bed
10. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	☐	☐	☐	☐

If you have a roommate or bed partner, ask him/her how often in the past month you have had...

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a) ...loud snoring	☐	☐	☐	☐
(b) ...long pauses between breaths while asleep	☐	☐	☐	☐
(c) ...legs twitching or jerking while you sleep	☐	☐	☐	☐
(d) ...episodes of disorientation or confusion during sleep	☐	☐	☐	☐
(e) Other restlessness while you sleep; please describe	☐	☐	☐	☐

_____

_____

Epworth Sleepiness Scale

Name: _____

Date: _____

Your age: (Yr) _____ **Your sex:** ❑ Male ❑ Female

How likely are you to doze off or fall asleep in the situations described below, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the most appropriate number for each situation:-

0 = would never doze
1 = Slight chance of dozing
2 = Moderate chance of dozing
3 = High chance of dozing

Situation	Chance of dozing
Sitting and reading	
Watching TV	
Sitting, inactive in a public place (e.g. a theatre or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in the traffic	
Total	

Score:

0-10	Normal range
10-12	Borderline
12-24	Abnormal

4.4 ESTIMATION OF SALIVARY LEVELS OF MELATONIN

"Traditionally, serum has been used for analysis of melatonin, but there are practical limitations with using this type of measurement in the home or the workplace, particularly when repeated samples are needed to estimate the time of the melatonin peak. Overnight or first-void urine specimens are also widely used, and although reliable for information on overnight melatonin production[107][108](Travis RC *et al* 2003; Schernhammer ES *et al* 2004). They do not provide information on the 24-hour variation in levels. For the purposes of this study, multiple samples of saliva provided a simple and reliable measure of daily patterns of melatonin levels[109][110] (Voultsios A *et al* 1997; Nagtegaal E *et al* 1998), which was more practical than repeated urine or serum samples for use 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)[111]".

4.4.1 Collection of saliva

The participants are given instructions about collection of saliva in sterile tube along with sterile cotton swabs. The night workers 30 out of 30 were agreed to sample their saliva, while in the work in their call center were advised to collect saliva samples on precise time 8 pm, 10 pm, 12 am, as instructed prior, with light level maintained constantly dim < 10 lux, such that endogenous secretion of melatonin was not suppressed. Soon after the collection of saliva cotton swabs were disposed safely and the samples were transported maintaining -20 degree c until they are stored in a deep freezer with -20 degree c. Saliva samples were frozen and then stored at -20°C until they were shipped frozen to TamilNadu Dr.MGR Medical University for melatonin levels determined by ELISA reader.

4.4.2 Storage of samples

A well maintained deep freezer with -20 degree c was available in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai. All the samples were stored in the deep freezer until estimation of salivary melatonin.

4.4.3 Estimation of salivary melatonin:

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 (refer photograph 2).**

Principles of assay:

"This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for MT has been pre-coated onto a microplate. Standard and samples are pippeted into the wells and any MT present is bound by the immobilized antibody. After removing any unbound substances, abiotin –conjucated antibody specific for MT is added to the wells. After washing, avidin conjucated Horshdish peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of MT bound in the initial step. The color development is stopped and the intensity of the color is measured by ELISA reader values plotted to get the MT concentration".

Contents of the kit

1. Wash Buffer
2. Biotin antibody and its diluent
3. HRP avidin and its diluent
4. Standard
5. TMB substrate
6. Stop solution
7. Sample Diluent

STATISTICAL ANALYSIS

The data collected were subjected to Statistical analysis using the software SPSS version 21.

Student'sT test was carried out to compare the means of variables between day worker sand shift workers.

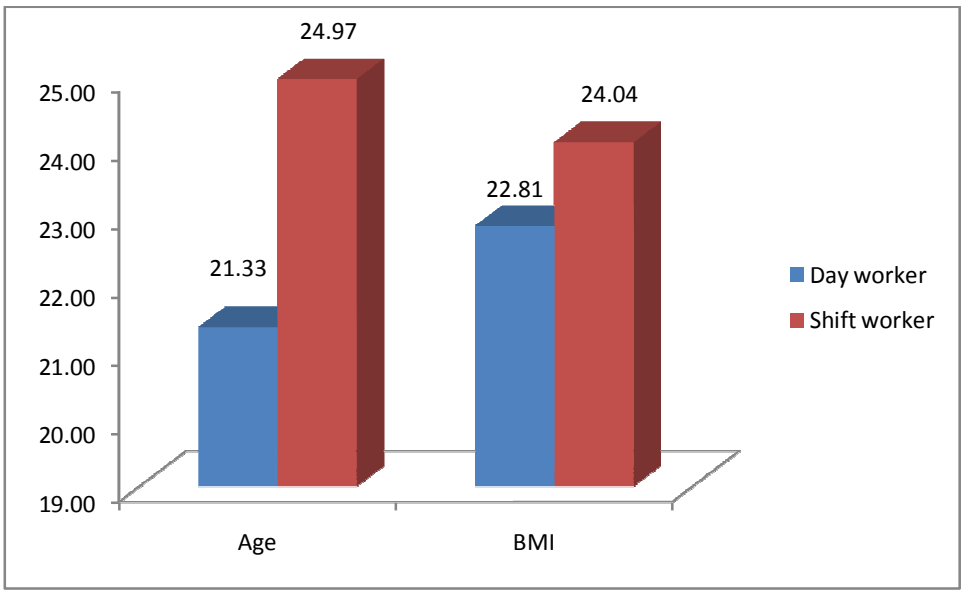
RESULTS

RESULTS

TABLE I			
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

TABLE II					
Comparison of Sleep Efficiency between Day worker and Shift worker					
Variable	Group	N	Mean	SD	P -Value
SLEEP EFFECIENCY	Day worker	30	91.52	3.71	.0001**
	Shift worker	30	83.81	7.38	
** P – Value < 0.001 Very Highly Significant					

The Sleep Efficiency percentage of shift worker shows a very highly significant decrease (**P-Value < 0.001) when compared to day worker


24.97
24.04
22.81
21.33
25.00
24.00
23.00
22.00
21.00
20.00
19.00
Age
BMI
Day worker
Shift worker

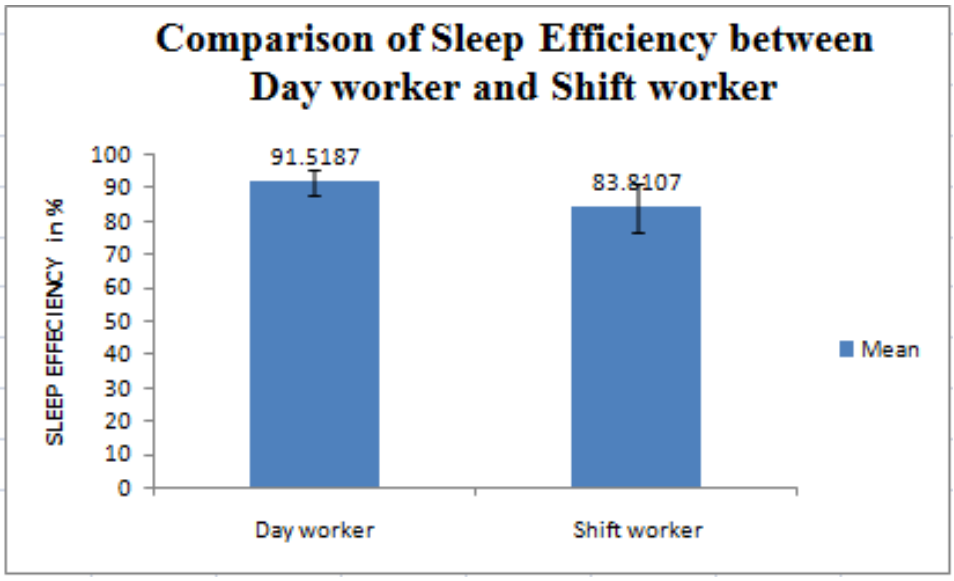
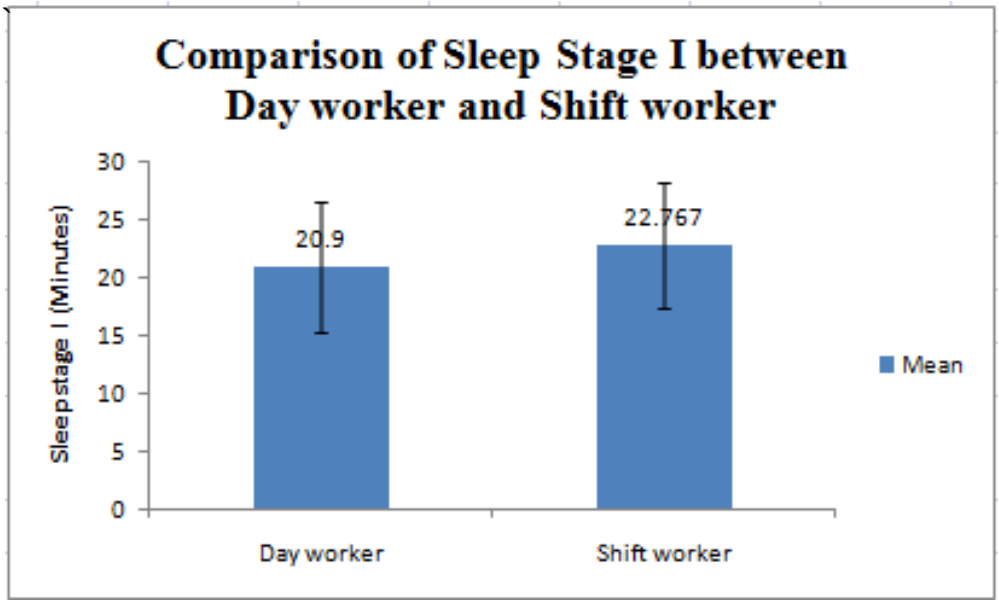

Comparison of Sleep Efficiency between Day worker and Shift worker
SLEEP EFFECIENCY in %
100
90
80
70
60
50
40
30
20
10
0
91.5187
83.8107
Day worker
Shift worker
Mean

TABLE III					
Comparison of Sleep Stage I between Day worker and Shift worker					
Variable	Group	N	Mean	SD	P -Value
Sleep stage I (Minutes)	Day worker	30	20.9	5.59	0.193
	Shift worker	30	22.77	5.38	
* P – Value < 0.05 Significant					

The Sleep stage I of shift worker shows a decrease but not significant (P-Value 0.193) when compared to day worker

TABLE IV					
Comparison of Sleep Stage II between Day worker and Shift worker					
Variable	Group	N	Mean	SD	P -Value
Sleep stage II (Minutes)	Day worker	30	203.67	14.93	0.617
	Shift worker	30	205.65	15.60	
* P – Value < 0.05 Significant					

The Sleep stage II of shift worker shows a decrease but not significant (P-Value 0.617) when compared to day worker


Comparison of Sleep Stage I between Day worker and Shift worker
Sleepstage I (Minutes)
30
25
20
15
10
5
0
20.9
22.767
Day worker
Shift worker
Mean

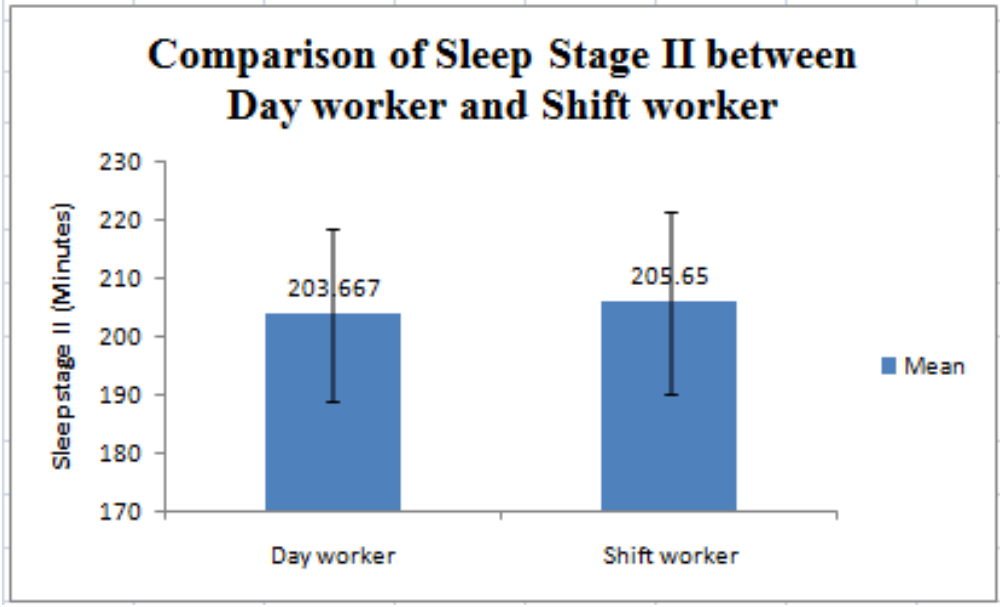
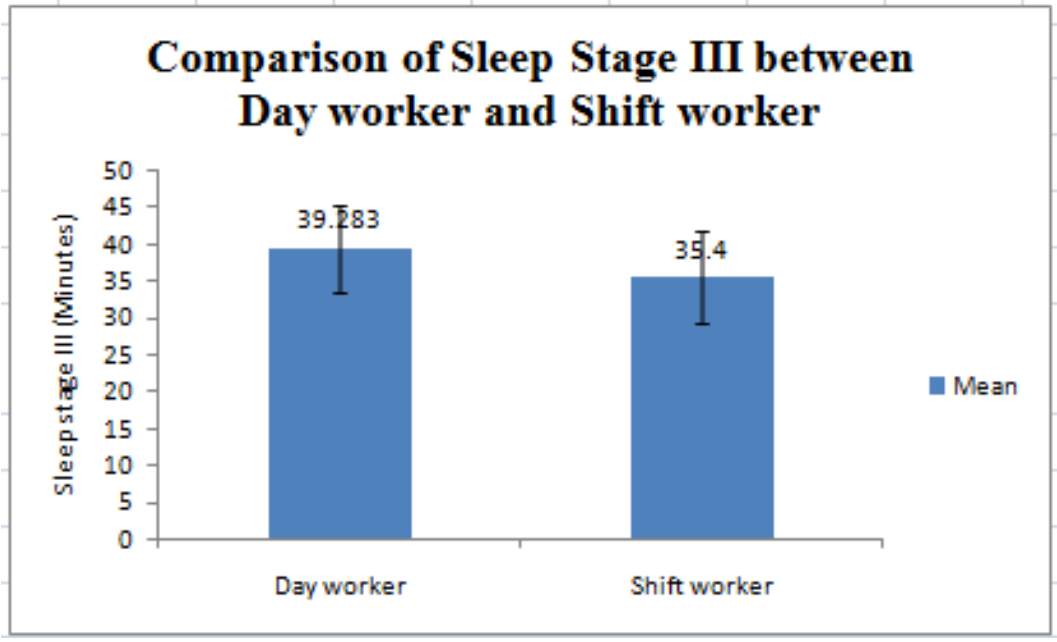

Comparison of Sleep Stage II between Day worker and Shift worker
Sleepstage II (Minutes)
230
220
210
200
190
180
170
203.667
205.65
Day worker
Shift worker
Mean

TABLE V					
Comparison of Sleep Stage III between Day worker and Shift worker					
Variable	Group	N	Mean	SD	P –Value
Sleep stage III (Minutes)	Day worker	30	39.28	5.99	0.017*
	Shift worker	30	35.40	6.25	
* P – Value < 0.05 Significant					

The Sleep stage III of shift worker shows a significant decrease (*P-Value) when compared to day worker

TABLE VI					
Comparison of Sleep Stage IV between Day worker and Shift worker					
Variable	Group	N	Mean	SD	P –Value
Sleep stage IV (Minutes)	Day worker	30	42	8.11	0.07
	Shift worker	30	38.13	8.49	
* P – Value < 0.05 Significant					

The Sleep stage IV of shift worker shows a decrease but not significant (P-Value 0.07) when compared to day worker


Comparison of Sleep Stage III between Day worker and Shift worker
Sleep stage III (Minutes)
50
45
40
35
30
25
20
15
10
5
0
39.283
35.4
Day worker
Shift worker
Mean

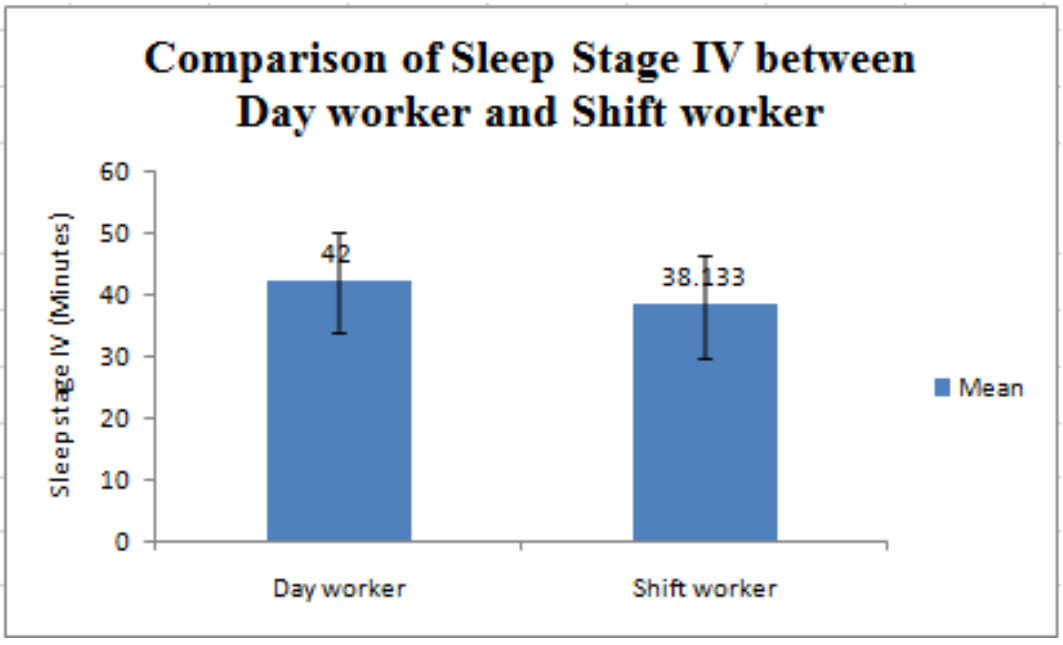
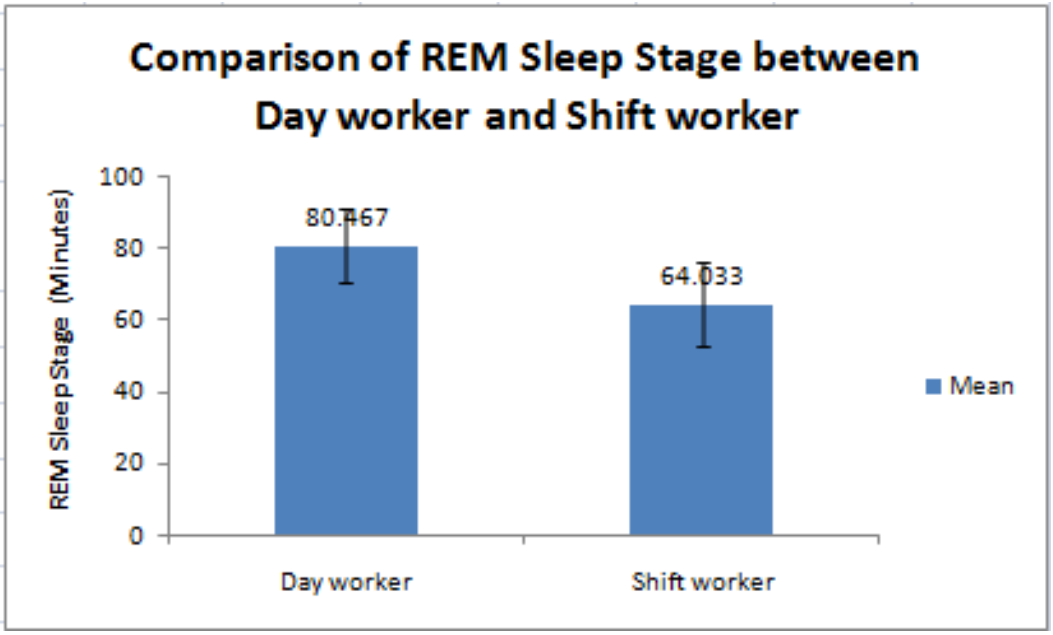

Comparison of Sleep Stage IV between Day worker and Shift worker
Sleep stage IV (Minutes)
60
50
40
30
20
10
0
42
38.133
Day worker
Shift worker
Mean

TABLE VII					
Comparison of REM Sleep Stage between Day worker and Shift worker					
Variable	Group	N	Mean	SD	P –Value
REM Sleep Stage (Minutes)	Day worker	30	80.47	10.10	.0001**
	Shift worker	30	64.03	11.58	
** P – Value < 0.001 Very Highly Significant					

The REM Sleep Stage of shift worker shows a very highly significant decrease (**P-Value) when compared to day worker

TABLE VIII					
Comparison of NAP onset latency between Day worker and Shift worker					
Variable	Group	N	Mean	SD	P –Value
NAP onset latency (Minutes)	Day worker	30	13.25	4.69	.0001**
	Shift worker	30	19.73	3.26	
** P – Value < 0.001 Very Highly Significant					

The NAP onset latency of shift worker shows a very highly significant increase (**P-Value) when compared to day worker


Comparison of REM Sleep Stage between Day worker and Shift worker
REM Sleep Stage (Minutes)
100
80
60
40
20
0
80.467
64.033
Day worker
Shift worker
Mean

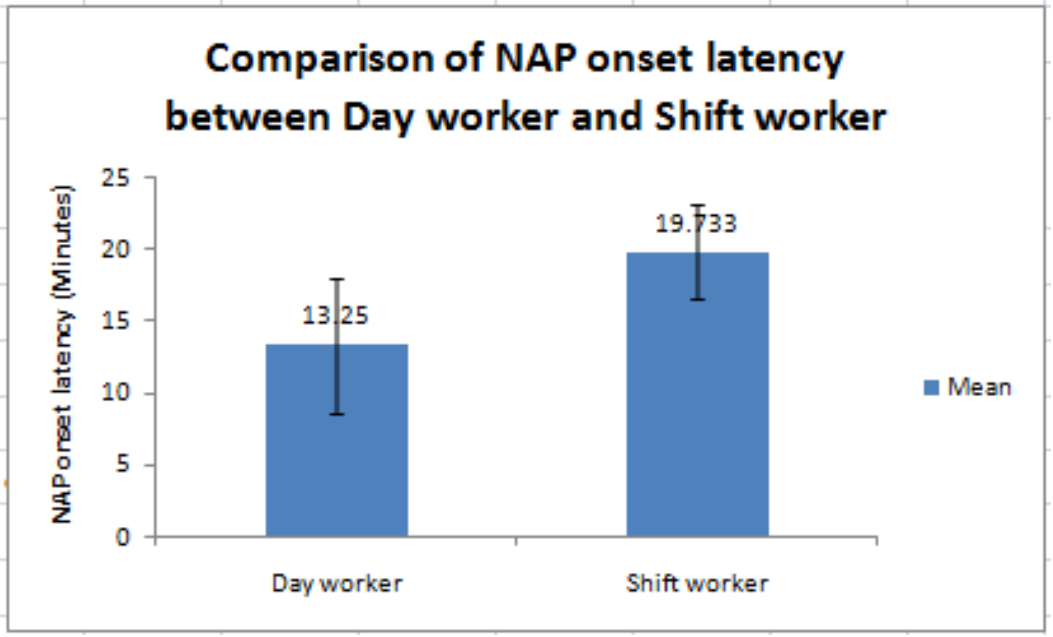

Comparison of NAP onset latency between Day worker and Shift worker
NAP onset latency (Minutes)
25
20
15
10
5
0
13.25
19.733
Day worker
Shift worker
Mean

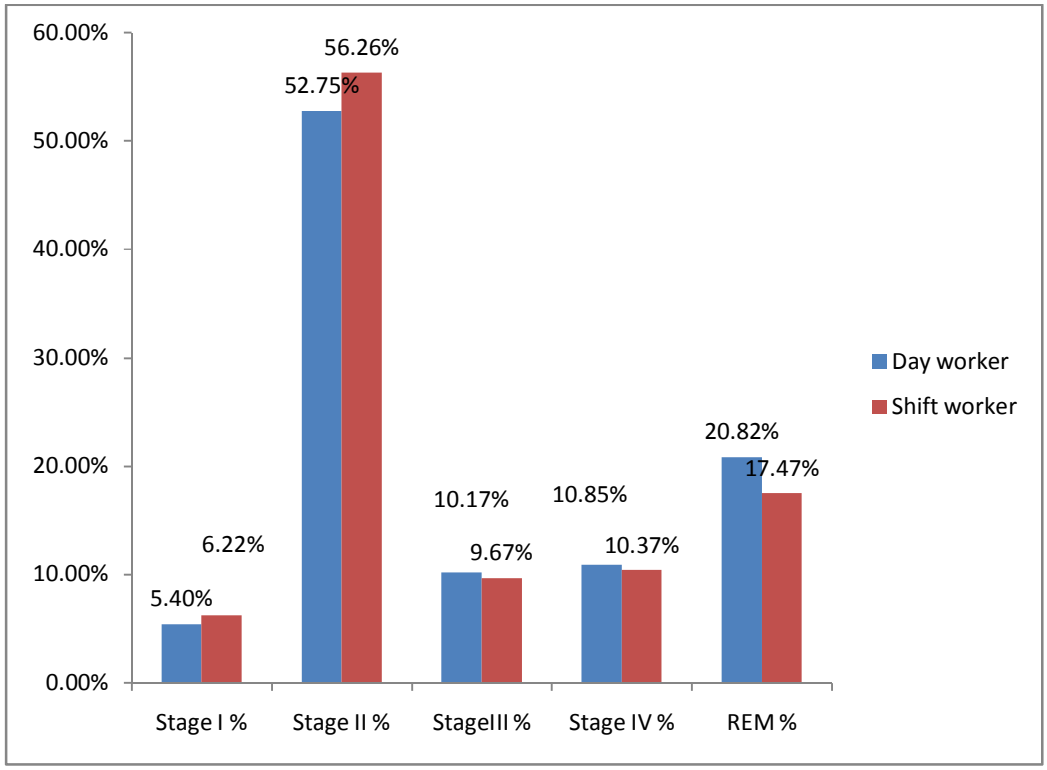
TABLE IX

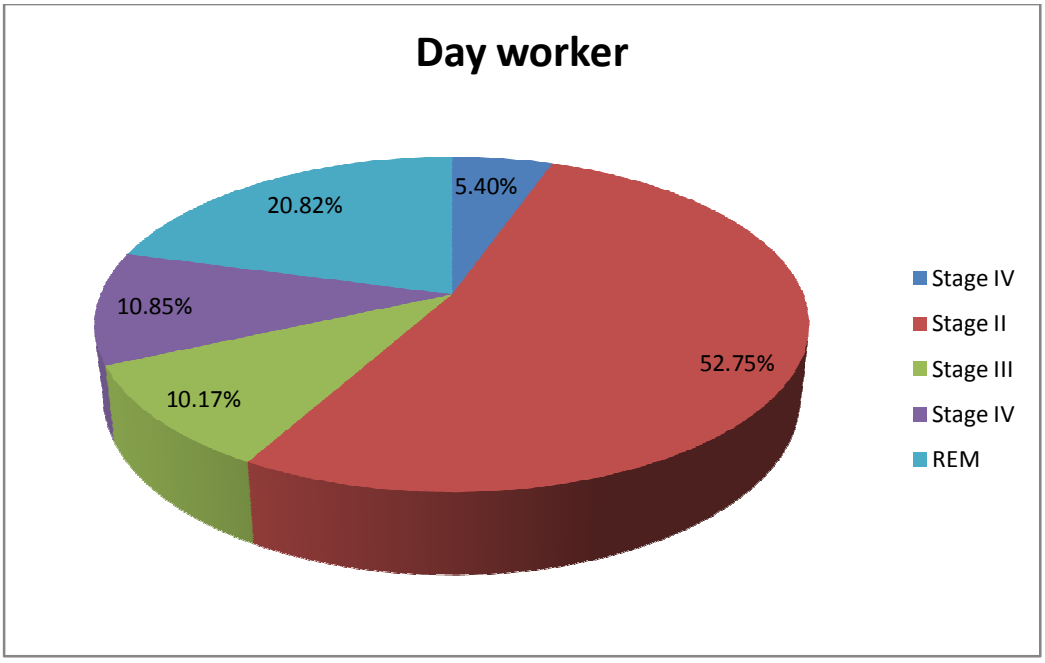
Comparison of Sleep Stage Percentage between Day worker and Shift worker

Variable	Group	Mean	SD	P -Value
Stage I % (Stage I/Total Sleep Time*100)	Day worker	5.40%	1.41%	0.030*
	Shift worker	6.22%	1.45%	
Stage II % (Stage II/Total Sleep Time*100)	Day worker	52.75%	3.58%	0.001*
	Shift worker	56.26%	4.07%	
Stage III % (Stage III /Total Sleep Time*100)	Day worker	10.17%	1.51%	0.224
	Shift worker	9.67%	1.68%	
Stage IV % (Stage IV/Total Sleep Time*100)	Day worker	10.85%	1.93%	0.350
	Shift worker	10.37%	2.04%	
REM % (REM/Total Sleep Time*100)	Day worker	20.82%	2.45%	0.0001**
	Shift worker	17.47%	2.89%	

* P – Value < 0.05 Significant

** P – Value < 0.001 Very Highly Significant


60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
5.40%
6.22%
52.75%
56.26%
10.17%
9.67%
10.85%
10.37%
20.82%
17.47%
Stage I %
Stage II %
StageIII %
Stage IV %
REM %
Day worker
Shift worker


Day worker
5.40%
52.75%
10.17%
10.85%
20.82%
Stage IV
Stage II
Stage III
Stage IV
REM

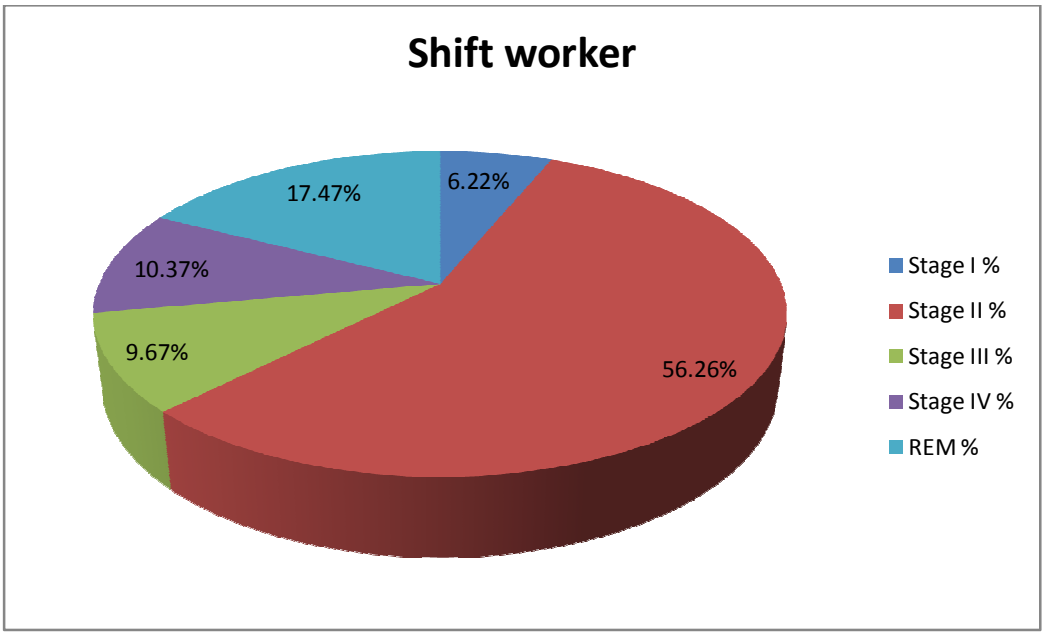

Shift worker
6.22%
56.26%
9.67%
10.37%
17.47%
Stage I %
Stage II %
Stage III %
Stage IV %
REM %

TABLE X

Comparison of NAP time between Day worker and Shift worker

Variable	Group	N	Mean	SD	P -Value
NAP time	Day worker	30	386.32	16.69	.0001**
	Shift worker	30	365.98	20.62	

** P – Value < 0.001 Very Highly Significant

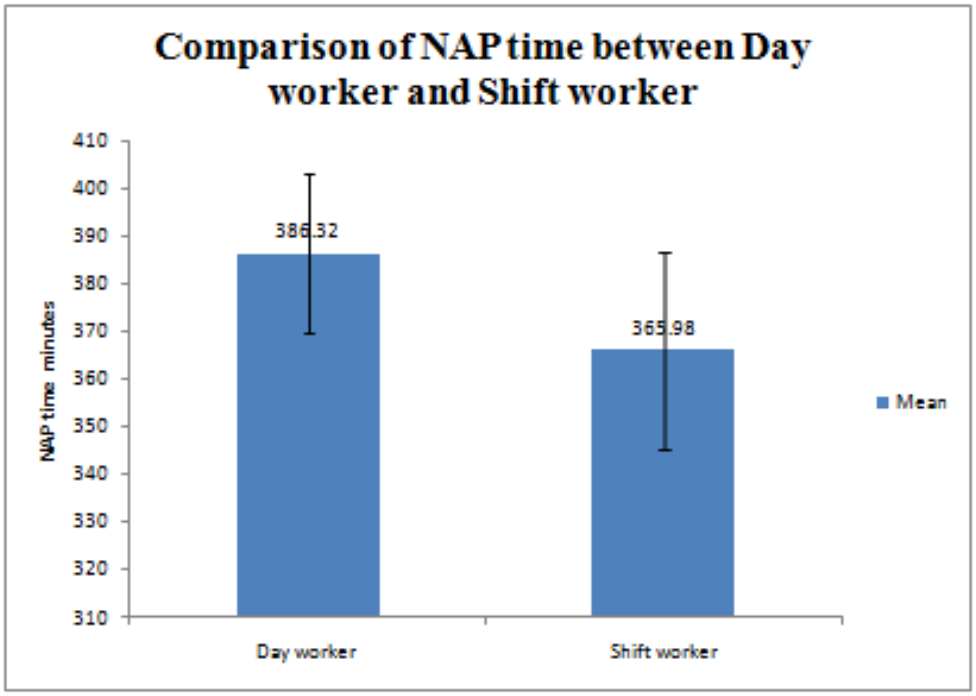
TABLE XI

Comparison of subjective sleep scores between Day worker and Shift worker

Variable	Subjects	Questionnaires	Mean	SD	P -Value
Subjective sleep scores	day worker	PSQI	0.77	1.33	0.000**
	shift worker	PSQI	4	2.69	
	day worker	ESS	6.23	2.21	0.009*
	shift worker	ESS	8.56	4.22	

* P value < 0.01 highly significant

** P – Value < 0.001 Very Highly Significant


Comparison of NAP time between Day worker and Shift worker
NAP time minutes
410
400
390
380
370
360
350
340
330
320
310
386.32
365.98
Day worker
Shift worker
Mean

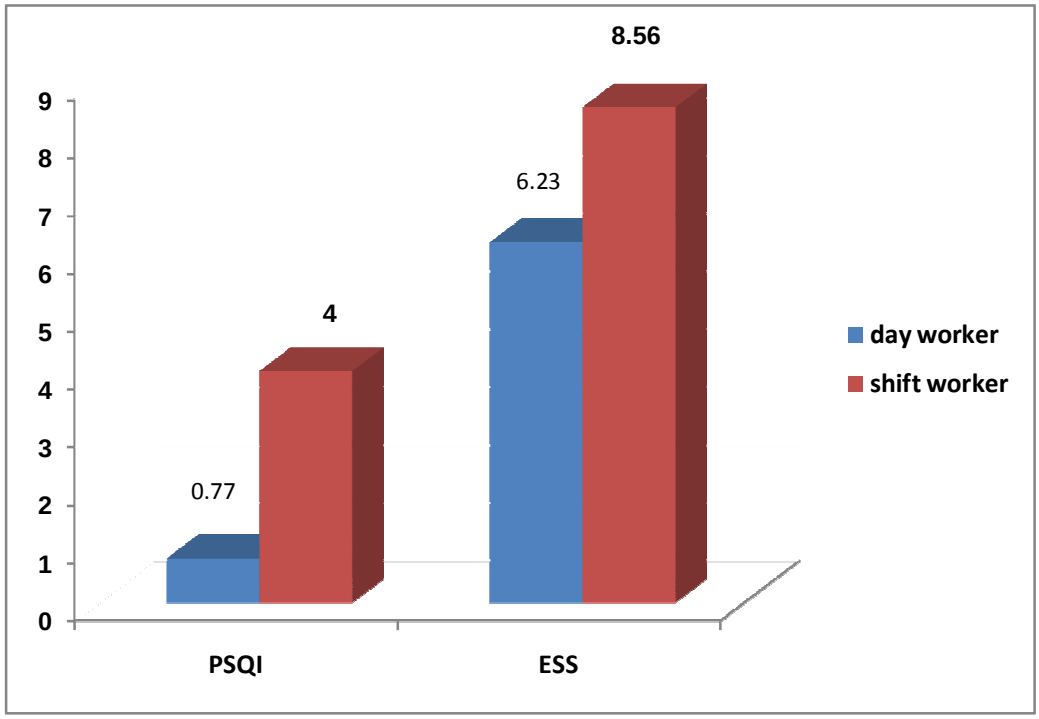
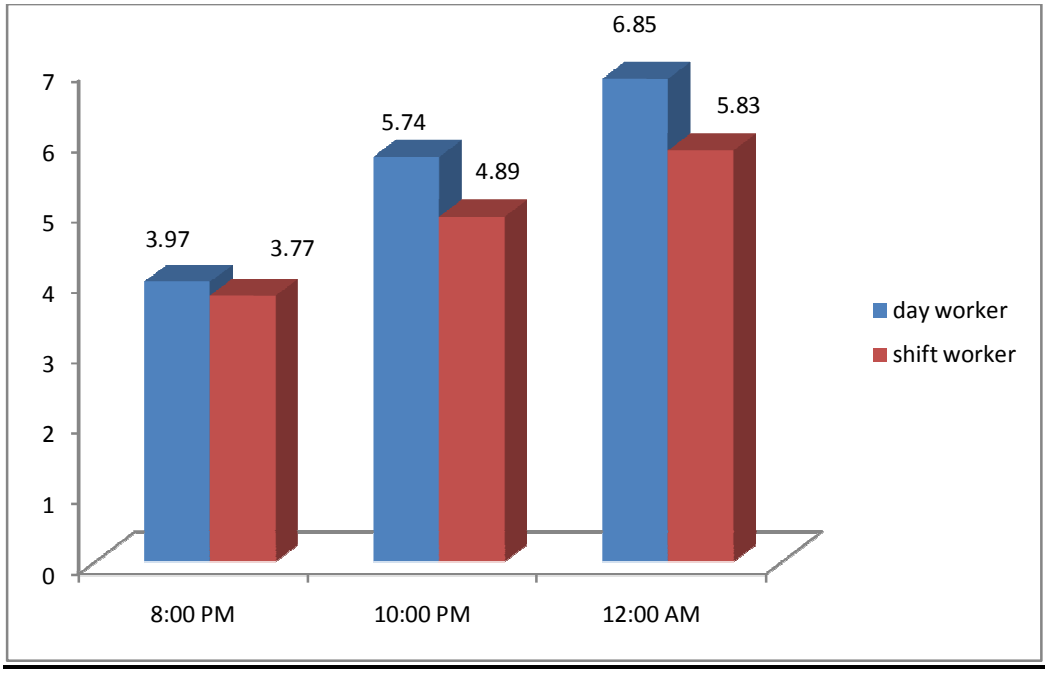

8.56
9
8
7
6
5
4
3
2
1
0
6.23
4
0.77
PSQI
ESS
day worker
shift worker

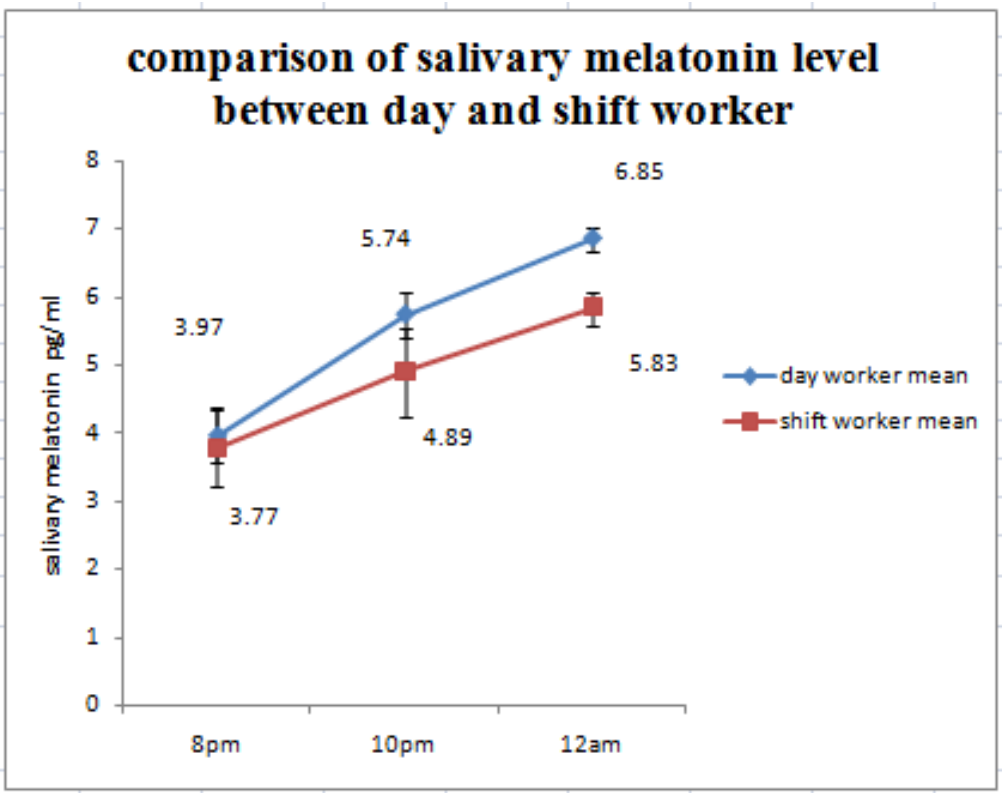
TABLE XII

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

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

** P – Value < 0.001 Very Highly Significant


6.85
5.83
5.74
4.89
3.97
3.77
7
6
5
4
3
2
1
0
8:00 PM
10:00 PM
12:00 AM
day worker
shift worker


comparison of salivary melatonin level between day and shift worker
salivary melatonin pg/ml
8
7
6
5
4
3
2
1
0
6.85
5.74
3.97
5.83
4.89
3.77
8pm
10pm
12am
day worker mean
shift worker mean

DISCUSSION

DISCUSSION

This study was done to record the sleep stages and find the salivary melatonin secreting pattern between day workers and rotating shift workers along with their subjective sleep scores. The mean age of the study groups were comparable 21.33±0.48 for day workers and 24.97±2.55 for shift worker as well as the body mass index for day worker (22.81±1.65) and shift worker (24.04±1.64). None of them were obese by means of BMI. Both the group persons were subjected to polysomnography for a minimum period of 6 hours to maximum period of 8 hours. The obtained data were analyzed statistically and independent sample T test was used to obtain the significance.

Scoring of sleep stages:

Sleep stages were interpreted with respect to the duration of each sleep stage, latency onset of sleep stage, total sleep duration and sleep efficiency. Total sleep time was recorded as the sum of all 30 second epochs of REM and non REM sleep. The mean total sleep time (NAP time) for day worker 386.32 ±16.69 minutes shift worker 365.98±20.62 minutes. These results were consistent with the study by Akersted T *et al* 1991[112] and Tepas DI *et al* 1981[113].

The mean Sleep Efficiency percentage of shift worker shows a very highly significant decrease 83.81±7.38% when compared to day worker 91.52±3.71 %. This may be a cause of sleep maintenance insomnia in shift workers in whom internal circadian rhythm is desynchronized. However SE did not differ between shifts in the study done by Akersted T *et al* 1991 and Dahlgren K *et al* 1981[114].

Sleep stage I of shift worker 22.77±5.38 minutes of mean value shows a decrease but not significant when compared to day worker 20.9±5.59 minutes. Sleep stage II of shift worker shows a decrease but not significant, when compared to day worker 203.67±14.93 minutes, shift worker 205.65 ± 15.60 minutes. Similar decrease in stage II was obtained in the study done by Akersted T *et al* 1991. Excessive day time sleepiness is associated with increase in sleep stage I increased arousal frequency leading to loss of continuity in sleep, decrease in duration of slow wave sleep and REM sleep which is consistent with the results of the study (Alexandros N.Vgontzas *et al*., 1994) [115].

Sleep stage III of shift worker shows a significant decrease when compared to day worker, 39.28 ±5.99 minutes, shift worker 35.40±6.25 minutes. Sleep stage IV of shift worker shows a decrease but not significant when compared to day worker, 42±8.11 minutes, shift worker

38.13±8.49 minutes. These slow wave sleep were found to be decreased in the study done by Akersted T *et al* 1991 and Torsvall L *et al* 1981[116] .

Rapid Eye Movement Sleep Stage of shift worker shows a very highly significant decrease when compared to day worker 80.47±10.10 minutes, shift worker 64.03±11.58 minutes. Similar results were obtained in the study done by Akersted T *et al* 1991and Matsumoto K *et al* 1978[117].

NAP onset latency is defined as the duration of time between lights out and sleep onset, defined by consecutive epochs of stage I or first stage of deeper NREM stage. The NAP onset latency measured in minutes for shift worker shows a very highly significant increase when compared to day worker 13.25±4.69 minutes, and shift worker 19.73±3.26 minutes. This may be related to sleep onset insomnia as commonly reported in sleep clinics by employees. The result was consistent with the study done by Foret J *et al* 1972[118].

The mean percentage value of the sleep stages showed reduction in deep slow wave sleep and rapid eye movement stage, there by occupying the counter portion by the stage I & II as the following : Sleep stage I day worker 5.40%±1.41%, shift worker 6.22%±1.45%, Sleep stage II day worker 52.75% ±3.58%, shift worker 56.26% ± 4.07%, Sleep stage III

day worker 10.17% ± 1.51%, shift worker 9.67% ±1.68%, , Sleep stage IV day worker 10.85%±1.93%, shift worker 10.37% ± 2.04%, stage REM day worker 20.82% ±2.45%, shift worker 17.47% ± 2.89%. Similar results were obtained in the study done by Torsvall L *et al* 1989[119]. The slow wave sleep is reduced and the part is occupied by stage 1 & 2 of sleep.

Subjective sleep scores for shift workers 4±2.69 for PSQI were higher than for the day worker 0.77± 1.33, as well as higher scores for ESS obtained for shift worker 8.56±4.22 as compared to day worker 6.23±2.21, showing poor quality of sleep for shift workers and more prone for dozing during the period when he usually is awake. These results were consistent with the study by Tilley AJ *et al* 1982[120].

Melatonin secretory pattern in saliva showed a lower trend for shift worker as evidenced by salivary melatonin ELISA assay Shift worker 8pm 3.77±0.56 10 pm 4.89±0.66 12 am 5.83 ± 0.25 (in pg/ml) as compared to day worker 8pm 3.97 ±0.42 10pm 5.74± 0.34, 12 am 6.85±0.18 (in pg/ml). The limitation of the study is base line secretion of melatonin prior to 20:00 and melatonin concentration after 00:00 were not measured. Therefore DLMO occurred beyond this interval could have been missed. A study done by gooneratne *et al*[121] 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 shift workers during dim light may be an association with disrupted circadian rhythms in rotating shift workers, which are further supported by the melatonin results.

There was no significant difference in the melatonin level at 8 pm, between shift and day worker. But melatonin levels at 10 pm and 12 am were found to be statistically significant ($p < 0.001$). Melatonin levels in those working rotating shifts were abnormal, with low levels. This pattern possibly suggests that fast-rotating shifts (6 days, 6 nights, a day off between switch over) might not have allowed sufficient time for adaptation to occur. The consequences of this are unheard of.

So it is unclear from this study whether rotating 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 rotating 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 years 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 only one point in time is measured, because self-report may take into account average exposure instead of a single snapshot in time[122] (Teschke K *et al* 2002).

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 chance or potential confounders such as BMI, alcohol intake or co morbidities as alternate explanations.

Overnight or first-void urine specimens are also widely used, and although reliable for information on overnight melatonin production [123124](Travis RC *et al* 2003; Schernhammer ES *et al* 2004), they do not provide information on the 24-hour variation in 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[125126] (Voultsios A *et al* 1997; Nagtegaal E *et al* 1998), which was more practical than repeated urine or serum samples for use 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)[127]. 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 [128] (Lowden A *et al* 2004). Koller *et al*[129] measured light exposure in permanent night-shift workers for a period of 48 hours, while Dumont *et al* 2001[130] 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[131] 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 [132](Demoss C *et al* 2004) 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[133]. These results contribute possibility of data for the study of light at-night exposure in the workplace. They demonstrated that larger measurement studies are feasible, and that rotating shift work may be associated with abnormal melatonin levels and irregular light exposure patterns, so its use as a surrogate is supported by the 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 rotating shift and straight 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[134](Barger LK *et al* 2004),

or exposure to bright light during work and wearing dark glasses after work to improve adaptation to shift work [135](Boivin DB *et al* 2002). The low levels of melatonin during sleep may be addressable with exogenous melatonin[136] (Arendt J *et al* 2005), though no long-term safety, dosing or formulation data yet exist.

CONCLUSION

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The following conclusions have been derived from the study:

- The Sleep Efficiency percentage of shift worker shows a significant decrease when compared to day worker
- The Sleep stage I, II and IV of shift worker shows a decrease but not significant when compared to day worker indicate
- The Sleep stage III of shift worker shows a significant decrease when compared to day worker indicate shift worker does not undergo a satisfactory deep sleep, which make them feel unrefreshed while in wake period after day time sleep.
- The REM Sleep Stage of shift worker shows a significant decrease when compared to day worker
- While in the view of percentage wise occupation of sleep stages, rapid eye movement stage and slow wave sleep were reduced and the counterpart occupied by the stage I , II and III.
- The NAP onset latency of shift worker shows a significant increase when compared to day worker denoting the cumulative lack of sufficient sleep in shift workers.
- Subjective self rated sleep scores (PSQI & ESS) showed increased values for shift workers stating that their dozing moments in active wake period, due to inadequate sleep.

- Trend of melatonin secretion in dim light for shift workers were lower when compared to day worker indicating their misalignment of hormone for shift worker.

Further studies may be necessary to define accurate circadian phase of shift worker by assessing 24 hour melatonin and also influence of other hormones on melatonin. In future association of memory, cognition and prospective well being can also be studied for shift worker for obtaining mechanisms underlying pathophysiology of the ill effects.

LIMITATION OF THE STUDY

- 24 hour serial assay of melatonin level was not performed, so our study could have missed the possible raise of trend of melatonin in shift workers in their day time sleep.

- Only 15 individuals from each group were taken for salivary sample collection considering the cost of the ELISA kit.

- Sleeping environment and socio economic profile for shift workers are not same for everyone. Social, personal, domestic factors may influence their quality of sleep further then their work.

- Sleep quality was assessed subjectively which could not exclude personal scoring bias.

SUMMARY

SUMMARY

Globalization leads round the clock service providers (shift workers) vulnerable for circadian rhythm sleep disorders. It should be considered in the differential diagnosis of patients presenting with symptoms of insomnia or excessive sleepiness. Delayed sleep phase type and advanced sleep phase type circadian rhythm sleep disorders may be associated with other types of sleep disorders, creating the diagnosis and treatment even more difficult for health care provider. For successful management of circadian rhythm sleep disorders, one must obtain as accurate measure of circadian timing as possible. Sleep onset time (determined by sleep diary or actigraphy) can be useful to determine circadian phase (dim light melatonin onset occurs approximately 2 hours after sleep onset) in the clinical setting.

Health education, awareness of sleep physiology, behavioral interventions such as sleep hygiene, enforcement of stable sleep and wake times, prevention of exposure for light at the wrong time of the day and exposure to light at the correct time of day are the basic approach for all the patients. For the treatment of DSPT and nonentrained type, administration of melatonin may be useful. However, use of melatonin for the treatment of circadian rhythm sleep disorders has not been

approved by the U.S Food and Drug Administration, and vascular and endocrine adverse effects need to be taken into account, particularly in patients who are at increased risk.

Bright light and melatonin can be used successfully in the treatment of circadian rhythm sleep disorders. However, appropriate timing of the treatment is crucial for the effect. An estimation of the Patient's circadian phase is therefore important before the treatment is started. In clinical practice, a careful patient history may give sufficient information in order to estimate the circadian phase. Appropriate timing of melatonin administration is about 12hour after bright light treatment.

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ANNEXURES

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax: 044 25363970

CERTIFICATE OF APPROVAL

To

Dr.N.S.Sasikumar,
Postgraduate
Institute of Physiology and Experimental Medicine,
Madras Medical College, Chennai-3.

Dear **Dr.N.S.Sasikumar,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Study of pattern of secretion of Melatonin in saliva and sleep parameters in shift workers "** No.25042014

The following members of Ethics Committee were present in the meeting held on 11.03.2014 conducted at Madras Medical College, Chennai-3.

1. Dr. C.Rajendran, M.D, -- Chairperson
2. Prof. Kalaiselvi, M.D, -- Member Secretary
   Vice Principal, MMC, Ch-3
3. Prof. Nandhini, M.D, -- Member
   Inst. of Pharmacology, MMC, Ch-3
4. Prof.Bhavani Sankar, M.S, -- Member
   Prof & HOD General Surgery, MMC, Ch-3
5. Prof.V.Padmavathi, M.D, -- Member
   I/c. Director of Pathology, MMC, Ch-3
6. Thiru. S. Govindasamy, BA., BL -- Lawyer
7. Tmt.Arnold Saulina, MA MSW -- Social Scientist
8. Thiru.S.Ramesh Kumar, -- Lay Person
   Administrative Officer, MMC, Ch-3.

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

21/4/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

21.4.14

INFORMED CONSENT FORM

Title of the study: "Study of pattern of secretion of Melatonin in saliva and sleep parameters in shift workers as compared to day workers"

Name of the Participant:

Name of the Principal Investigator: Dr.N.S.Sasikumar

Name of the Institution:

Institute of Physiology and Experimental Medicine,

Madras Medical College and Govt. General Hospital,

Chennai - 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in

"Study of pattern of secretion of Melatonin in saliva and sleep parameters in shift workers as compared to day workers"

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.

4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past _____month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.
11. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

12. I have understood that my identity will be kept confidential if my data are publicly presented.
13. I have had my questions answered to my satisfaction.
14. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____

Date_____

PROFORMA

1. Name :
2. Age:
3. Sex:
4. Address :
5. Occupation :
6. Complaints/duration:
7. History of present illness:
8. History of any hearing problem after the onset of epilepsy?
9. Past history:
10. History of any drug intake
11. History of associated illness:
    a. Diabetes
    b. Hypertension
    c. Ischemic heart disease
    d. Respiratory diseases
    e. Renal diseases

Investigations:

Salivary melatonin levels

EXAMINATION

General examination:

Temperature: Pulse rate Blood pressure:

Systemic examination:

Cardiovascular system:

Respiratory system:

Gastrointestinal system:

Central nervous system:

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு :

பாலிசோம்னோகிராஃபி மற்றும் உமிழ்நீர் மெலடோனின் அளவை ஷிஃப்ட் முறை பணியாளர்களிடம் ஆராய்ந்து அறிதல்

பெயர்:

வயது:

பாலினம்: ஆண் / பெண்

பங்கு பெறுபவர் அடையாள எண்:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கமும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தை தெரிவிக்கிறேன்.

எனது பாலிசோம்னோகிராஃபி மற்றும் உமிழ்நீர் மெலடோனின் அளவை பரிசோதனை செய்ய முழு சம்மதம்.

இந்த ஆராய்ச்சியில் யாருடைய நிர்பந்தமுமின்றி சொந்த விருப்பத்தின் பேரில் சம்மதிக்கிறேன்.

இந்த ஆராய்ச்சியில் இருந்து நான் எந்த நேரமும் பின் வாங்கலாம் என்றும், அதனால் எந்த பாதிப்பும் எற்படாது என்பதையும் புரிந்து கொண்டேன்.

நான் தூக்கம் குறித்த இந்த ஆராய்ச்சியின் விவரங்கள் கொண்ட தகவல்களை பெற்றுக்கொண்டேன்.

உமிழ்நீர் மெலடோனின் பரிசோதனைக்கு எனக்கு பஞ்சு மூலம் உமிழ்நீர் எடுக்க சம்மதிக்கிறேன். உமிழ்நீர் எடுக்கும் போது வலி, அரிப்பு, மயக்கம், போன்ற பின் விளைவுகள் எற்படலாம் என்று தெரிந்து கொண்டேன்.

நான் என்னுடைய சுய நினைவுடன் மற்றும் முழு சம்மதத்துடன் இந்த ஆராய்ச்சிக்கு என்னை பரிசோதிக்க சம்மதிக்கிறேன்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்ப்பாளர் கையொப்பம்

நாள்:

இடம் :

PROFORMA

1. Name :
2. Age:
3. Sex:
4. Address :
5. Occupation :
6. Complaints/duration:
7. History of present illness:
8. History of any hearing problem after the onset of epilepsy?
9. Past history:
10. History of any drug intake
11. History of associated illness:
    a. Diabetes
    b. Hypertension
    c. Ischemic heart disease
    d. Respiratory diseases
    e. Renal diseases

Investigations:

Salivary melatonin levels

EXAMINATION

General examination:

Temperature: Pulse rate Blood pressure:

Systemic examination:

Cardiovascular system:

Respiratory system:

Gastrointestinal system:

Central nervous system:

MEDICAID SLEEP - LAB Industrial Area Phase - II Chandigarh

PT./MASTER ID : D 05 / 5
PATIENT NAME : N. ARUNPANDIAN
AGE : 21 Y / M
ADDRESS : chennai

TEST DATE / TIME : 1-July-2014, 00:00:00
REC DURATION : 07:14:00
DOCTOR NAME : SASIKUMAR
REFD BY : Dr. SASIKUMAR

SleepCare Analysis Report

RESPIRATION DATA

Variable(s)	NREM	REM
Obstructive Apnea	0	0
Mixed Apnea	0	0
Central Apnea	0	0
Hypopnea	9	0
Resp. DisOrder Index	02.51	00.00

SPO2 DATA

Variable(s)	Values	Units
Desaturation Index	.93	Per Hour
Total Number	18	Count
Avg. Saturation	97	(%)
Avg. Desaturation	10	Secs
Min Value	30	Count

S-STAGE VALUES

Variable(s)	% of TST	TOTAL MIN
SS - I	7.59	29
SS - II	57.85	221
SS - III	7.85	30
SS - IV	9.16	35
SS - AWAKE	11.98	52
Rapid Eye Mov.	17.54	67
Non -REM	82.46	315

POSITIONS DATA

Variable(s)	Time Spent (%)	Apnea/Hypo.	RDI
BODY - S	100.00	9	02.51
BODY - P	00.00	0	00.00
BODY - L	00.00	0	00.00
BODY - R	00.00	0	00.00
Total %age	100.00	9	02.51

CONCLUSION

Variable(s)	Value	Units
Start Time	00:00:00	HH:MM:SS
End Time	07:14:00	HH:MM:SS
NAP TIME	382	Minutes
NAP STATUS	88.02	(%)
NAP Onset Latency	16.5	Minutes

MASTER CHART- DAY WORKER

S No	age	BMI	stage I	stage II	stage III	stage IV	REM	stage I%	stage II%	stage III%	stage IV%	Rapid Eye Mov.%	TST	NAP time	NAP status	NAP onset latency	PSQI	ESS	melatonin level		
																			8:00 PM	10:00 PM	12:00 AM
1	21	20.24	25	191	54	46	81.5	6.29%	48.05%	13.58%	11.57%	20.50%	397.5	397.5	91.27	5	0	13	3.5	4.8	6.6
2	21	22.95	19.5	201.5	40	48	82.5	4.98%	51.47%	10.22%	12.26%	21.07%	391.5	391.5	92.23	12.5	0	3	3.8	5.5	7.1
3	22	23.53	34	220	40	32	71	8.56%	55.42%	10.08%	8.06%	17.88%	397	397	89.92	14	1	7	3.7	5.8	6.9
4	21	23.60	31.5	195	40	52	74.5	8.02%	49.62%	10.18%	13.23%	18.96%	393	393	75.6	18	1	4	3.6	5.7	6.7
5	21	21.30	29	221	30	35	67	7.59%	57.85%	7.85%	9.16%	17.54%	382	382	88.02	16.5	5	8	4	5.6	7
6	22	20.66	13.5	191	39	43	91	3.58%	50.60%	10.33%	11.39%	24.11%	377.5	377.5	92.52	7.5	5	7	3.9	5.9	6.8
7	21	20.76	18	195	44	56	85	4.52%	48.99%	11.06%	14.07%	21.36%	398	398	89.54	14	0	6	3.8	6	6.7
8	22	22.68	12.5	184	46	44.5	93.5	3.29%	48.36%	12.09%	11.70%	24.57%	380.5	380.5	90.38	6	0	6	4.2	5.5	6.8
9	21	21.71	28	180	43.5	46	64.5	7.73%	49.72%	12.02%	12.71%	17.82%	362	362	95.89	14	0	8	3.6	5.8	6.9
10	22	25.28	18	202.5	37.5	34.5	68.5	4.99%	56.09%	10.39%	9.56%	18.98%	361	361	95.76	7	0	8	3.8	5.6	7.1
11	21	22.95	21.5	216.5	32	29.5	63	5.93%	59.72%	8.83%	8.14%	17.38%	362.5	362.5	94.03	6.5	2	8	4.6	5.7	6.6
12	21	22.48	28	223.5	42	32.5	79	6.91%	55.19%	10.37%	8.02%	19.51%	405	405	90	8.5	0	7	3.7	6	6.7
13	21	25.97	19	213	40	49	70.5	4.85%	54.41%	10.22%	12.52%	18.01%	391.5	391.5	91.15	17.5	2	4	4.7	6.2	6.8
14	22	24.51	11.5	202	42.5	39	70.5	3.15%	55.27%	11.63%	10.67%	19.29%	365.5	365.5	93.24	18	0	6	4.8	5.9	6.9
15	21	25.10	15.5	198.5	31	37	102	4.04%	51.69%	8.07%	9.64%	26.56%	384	384	91.43	14	2	4	3.8	6.1	7.2
16	21	22.68	26	201	41.5	43	88.5	6.50%	50.25%	10.38%	10.75%	22.13%	400	400	93.35	15	0	5			
17	22	23.59	16	172	43	39	91	4.43%	47.65%	11.91%	10.80%	25.21%	361	361	94.63	15.5	0	3			
18	22	22.04	25.5	203	28	34	87	6.75%	53.77%	7.42%	9.01%	23.05%	377.5	377.5	91.29	9.5	0	4			
19	21	21.80	19	182	48	61	91	4.74%	45.39%	11.97%	15.21%	22.69%	401	401	91.66	7.5	0	9			
20	21	23.53	14.5	216.5	38	26	90.5	3.76%	56.16%	9.86%	6.74%	23.48%	385.5	385.5	95.54	10	0	6			
21	22	23.60	19	202	31	43	70.5	5.20%	55.27%	8.48%	11.76%	19.29%	365.5	365.5	90.25	16.5	0	4			
22	21	20.62	16.5	219	40.5	39	77.5	4.20%	55.80%	10.32%	9.94%	19.75%	392.5	392.5	92.57	12	1	8			
23	21	22.06	22	212	36	32	75	5.84%	56.23%	9.55%	8.49%	19.89%	377	377	93.66	18	0	5			
24	21	24.02	17	210	33	41	68	4.61%	56.91%	8.94%	11.11%	18.43%	369	369	95.6	22	1	8			
25	22	24.39	19.5	232	38	48	79	4.68%	55.70%	9.12%	11.52%	18.97%	416.5	416.5	88.52	22.5	0	6			
26	21	25.97	24.5	199	49	50.5	82	6.05%	49.14%	12.10%	12.47%	20.25%	405	405	90.5	17	0	4			
27	22	23.51	20	224	37	42.5	91.5	4.82%	53.98%	8.92%	10.24%	22.05%	415	415	92.53	13.5	0	6			
28	21	21.61	21	218	34	49	86.5	5.14%	53.37%	8.32%	12.00%	21.18%	408.5	408.5	89.49	17	1	4			
29	21	21.16	22	200	36	48	90	5.56%	50.51%	9.09%	12.12%	22.73%	396	396	93.95	10.5	1	9			
30	21	20.07	20	185	44	40	82	5.39%	49.87%	11.86%	10.78%	22.10%	371	371	91.04	12	1	7			

MASTER CHART- SHIFT WORKER

S No	age	BMI	stage I	stage II	stage III	stage IV	REM	stage I%	stage II%	stage III%	stage IV%	Rapid Eye Mov.%	TST	NAP time	NAP status	NAP onset latency	PSQI	ESS	melatonin level		
																			8:00 PM	10:00 PM	12:00 AM
1	28	23.46	24	204	15	20.5	45	7.78%	66.13%	4.86%	6.65%	14.59%	308.5	308.5	67.95	9	1	2	3.5	5	5.7
2	29	23.80	20.5	205.5	38	46	62.5	5.50%	55.17%	10.20%	12.35%	16.78%	372.5	372.5	80.54	18	3	5	2.9	4.8	6.1
3	22	24.74	36	222	38	30	43	9.76%	60.16%	10.30%	8.13%	11.65%	369	369	89.89	19	7	11	3	3.8	5.8
4	25	24.39	32.5	198	38	49	52	8.80%	53.59%	10.28%	13.26%	14.07%	369.5	369.5	74.57	22	3	13	3.1	4.2	5.6
5	23	22.96	30.5	224	28.5	33	57	8.18%	60.05%	7.64%	8.85%	15.28%	373	373	91.31	18	4	1	3.2	4.4	5.9
6	24	24.16	15.5	194	37	40	69	4.36%	54.57%	10.41%	11.25%	19.41%	355.5	355.5	89.21	17.5	6	1	4	5.8	5.7
7	25	20.80	21	197	41	54	75	5.41%	50.77%	10.57%	13.92%	19.33%	388	388	86.9	19	2	11	3.8	5.7	6.2
8	29	24.09	14.5	188	43	42	81.5	3.93%	50.95%	11.65%	11.38%	22.09%	369	369	82.37	20	4	4	4.2	5.5	5.9
9	28	25.40	30	183	41	44	53.5	8.53%	52.06%	11.66%	12.52%	15.22%	351.5	351.5	84.6	22	4	12	3.8	4.3	6
10	21	24.96	19.5	205.5	35	31	57.5	5.60%	58.97%	10.04%	8.90%	16.50%	348.5	348.5	92.07	19.5	7	6	4	4.5	5.2
11	23	26.29	23.5	220	29	26.5	52	6.70%	62.68%	8.26%	7.55%	14.81%	351	351	89.88	16.5	4	10	3.9	4.8	5.8
12	21	24.91	30	227	39	29	54.5	7.91%	59.82%	10.28%	7.64%	14.36%	379.5	379.5	85.96	20.5	2	12	4.8	5.2	5.7
13	23	24.01	21	215	38	46	52.5	5.64%	57.72%	10.20%	12.35%	14.09%	372.5	372.5	86.73	18.5	3	10	3.8	4.1	5.8
14	27	22.86	13.5	205	39.5	36	59.5	3.82%	57.99%	11.17%	10.18%	16.83%	353.5	353.5	90.06	22	13	9	4.7	5.4	6.1
15	26	24.39	17.5	201.5	28	34	78	4.87%	56.13%	7.80%	9.47%	21.73%	359	359	88.97	23	4	12	3.9	5.8	6
16	23	24.39	28	204	38.5	40	77.5	7.22%	52.58%	9.92%	10.31%	19.97%	388	388	72.73	19.5	3	11			
17	23	25.97	19	175	40.5	36	76	5.48%	50.51%	11.69%	10.39%	21.93%	346.5	346.5	89.65	18.5	2	11			
18	26	24.98	28	206	26	31	76	7.63%	56.13%	7.08%	8.45%	20.71%	367	367	80.57	19	8	9			
19	30	24.51	21	186	45	57	80	5.40%	47.81%	11.57%	14.65%	20.57%	389	389	75.61	17	6	9			
20	30	27.05	17.5	188	35	24	71.5	5.21%	55.95%	10.42%	7.14%	21.28%	336	336	67.6	19.5	3	15			
21	25	26.03	21.5	205	29	40	59	6.06%	57.83%	8.18%	11.28%	16.64%	354.5	354.5	86.67	21.5	1	11			
22	23	22.41	19	223	38.5	35	47	5.24%	61.52%	10.62%	9.66%	12.97%	362.5	362.5	87.77	21.5	4	10			
23	24	23.51	24	215.5	33.5	30	64	6.54%	58.72%	9.13%	8.17%	17.44%	367	367	84.66	22.5	4	7			
24	26	22.48	19.5	214	30	38.5	57	5.43%	59.61%	8.36%	10.72%	15.88%	359	359	88.86	23.5	2	10			
25	24	24.74	21.5	235	35.5	45	64	5.36%	58.60%	8.85%	11.22%	15.96%	401	401	79.88	28.5	8	12			
26	24	21.83	22.5	180	42	41.5	58	6.54%	52.33%	12.21%	12.06%	16.86%	344	344	67.92	23	3	12			
27	23	21.94	21.5	226.5	35	38.5	80.5	5.35%	56.34%	8.71%	9.58%	20.02%	402	402	85.81	20	4	13			
28	23	21.99	26	222	32	45.5	75.5	6.48%	55.36%	7.98%	11.35%	18.83%	401	401	92.18	18.5	2	7			
29	24	21.08	23.5	210	32.5	44.5	74	6.11%	54.62%	8.45%	11.57%	19.25%	384.5	384.5	88.7	16	2	2			
30	27	27.01	21	190	41	36.5	68.5	5.88%	53.22%	11.48%	10.22%	19.19%	357	357	84.7	19	1	2			