Study of Biological Rhythms in Patients Suffering From Upper Intestinal Cancer

Dissertation submitted in partial fulfillment of requirements for the degree of Master of Philosophy

by

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CERTIFICATE

I certify that the thesis entitled "**Study of Biological Rhythms in Patients Suffering From Upper Intestinal Cancer**" submitted to the Mizoram University for the award of the degree of Master of Philosophy in Zoology by **J. Lalremruati** is a record of research work carried out during the period of 2016 - 2017 under my guidance and supervision, and that this work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other titles in this University or any other University or institution of higher learning.

Signature of the Supervisor

(AMIT KUMAR TRIVEDI)

ACKNOWLEDGEMENTS

First of all I would like to thank God for His grace and guidance throughout my work and giving me good health and knowledge to complete my work.

I would like to thank all my volunteers and authorities of the Nazareth Cancer Hospital, Chatlang and Mizoram State Cancer Institute, Zemabawk for allowing me to conduct my study with the patients, without their support this study was not possible to conduct.

I would also like to thank Head of the Department Prof. G. Gurusubramanian and other faculty members for their help during my work.

I thank my lab colleagues Ms. Zothanmawii Renthlei and Mr. Bijoy Krishna Borah for their support and valuable suggestions for my research work.

I express my sincere gratitude to my supervisor Dr. Amit Kumar Trivedi, Department of Zoology, Mizoram University for his guidance and support.

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DECLARATION OF THE CANDIDATE

I, J. Lalremruati, hereby declare that the subject matter of this dissertation is the record of work done by me, that the contents of this dissertation did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the dissertation has not been submitted by me for any other University or Institute.

This is being submitted to Mizoram University for the degree of Master of Philosophy in the Department of Zoology.

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Contents

Page Number

Certificate i

Declaration of the Candidate ii

Acknowledgements iii

General Introduction 1-13

SECTION 1: To Study Cardiovascular Rhythms in Cancer Patients 14 - 29

1. Introduction 14 - 16
2. Material and methods 17 - 18
3. Statistical analysis 19
4. Results 20 - 22
5. Discussion 23 - 25
6. References 26 - 29

SECTION 2: To Study Activity Rest Pattern in Cancer Patients 30 - 44

1. Introduction 30 - 33
2. Material and methods 34 - 36
3. Statistical analysis 37
4. Results 38 - 39
5. Discussion 40 - 41
6. References 41 - 44

SECTION 3: To Study The Sleep Quality in Cancer Patients 45 - 64

1. Introduction 45 - 48
2. Material and methods 49 - 51
3. Statistical analysis 52
4. Results 53 - 56
5. Discussion 57 - 59
6. References 60 - 64

APPENDICES 65

Papers Presentation at Seminars /Workshop / Conferences

Seminars / Training / Workshop / Conferences Participated

GENERAL INTRODUCTION

Biological rhythms are regular changes occurring in almost all types of living organisms, in form of some variable physiological processes, irrespective of whether it occurs at a cellular level, tissue level, structural level, organismal level or population level (Kleitman 1949). These rhythms allow the organisms to adapt and live with the environmental variables around them, which are due to the rotation of the earth around the sun. The biological rhythms are endogenous in origin and are controlled by internal biological clocks and are maintained in the absence of the environmental cues. For example: daily temperature cycle, sleep-wake cycle, food cycle and many hormonal cycles. In nature, it is difficult to determine whether the activity of an animal is due to a direct effect or that of an endogenous biological clock, as the biological rhythms can be masked by environmental factors.

The biological rhythms have four fundamental characteristics:

(1) Biological rhythms are endogenous in origin i.e., rhythms persist in the absence of environmental cues and provides the organism an innate ability to maintain periods of a particular length between biological functions.

(2) Biological rhythms are temperature compensated to avoid biological rhythms being governed by the sudden fluctuations in the weather conditions.

(3) Biological rhythms have the ability to be reset in order to maintain a relationship with environmental cues.

(4) Biological rhythms are an internal continuous monitor of the passage of time, allowing the organism to keep track of duration of time biologically.

In general, the biological rhythms are output of the biological clock, but it is not an absolute rule, as some cycles may be due to consequence of some other nonlinear system. In mammals, the whole system is synchronized and aligned with the environment through signals emanating from the suprachiasmatic nucleus (SCN), a region in the brain referred to as the "central clock" (Mohawk *et al.* 2012). The SCN receives direct light input from the retinae of the eye, which is the most dominant environmental cue to synchronize the circadian clock (Dibner *et al*. 2010). While "peripheral clocks," are present in almost all organs and individual cells in the body, and are synchronized by the central clock through humoral and/or nervous signals, but can also operate independently of central clock input (Buijs *et al.* 1999). Some peripheral clocks like liver clock, are strongly entrained by the time of feeding, and misalignment of feeding and the central clock leads to metabolic disorders (Mukherji *et al.* 2015a,b). Synchronization of the peripheral clock can occur in cell culture by treatment with certain chemicals (Balsalobre *et al*. 2000), or just by changing culture media (Yeom *et al.* 2010).

The molecular circadian clock is governed by several feedback loops that lead to 24-h oscillations of target gene expression, defined by their *amplitude* (height), *phase* (position), and *period* (length).The molecular regulation of the circadian clock constitutes interlocking feedback loops involving cyclic gene products that control transcription by means of negative and positive regulation of 'clock' genes and proteins. The specific transcriptional/translational feedback loop components differ between phylogenetic kingdoms (Lakin2006). In mammals, cell-autonomous circadian clocks are generated by a autoregulatory transcriptional translational feedback loop composed of the transcriptional activators CLOCK and BMAL1, and their target genes *PERIOD* (*PER1* and *PER2*) and *CRYPTOCHROME* (*CRY1* and *CRY2*). The *PER* and *CRY* are able to form a repressor complex that interacts with CLOCK/BMAL1 to inhibit their own transcription (Reppert *et al*.

2002; Takahashi *et al*. 2008). Post-translational events that modulate protein half-life and sub-cellular localization appear to contribute significantly to circadian oscillations. Several kinases and phosphatases regulate the time, precision and function of the circadian clock (Gallego *et al.* 2007; Vanselow *et al.* 2010).

In mammals, the pineal gland is a one of the major component of the endocrine system that participates in time measurement and allows them to respond to the annual changes in photoperiod. Hormone melatonin, the product synthesizes and releases by the pineal gland, is secreted only during the dark period of the light/dark cycle, independently of whether the animal is diurnal or nocturnal. Duration of the nocturnal melatonin peak is proportional to the length of the night. The SCN is able to processes the photoperiodic information through these changes in duration of melatonin synthesis (Prendergast *et al*. 2002; Pévet 2003). Melatonin also modulates secretion of reproductive hormones by the anterior pituitary gland and therefore regulates the activity of the pars tuberalis in mammals (Dardente *et al.* 2010).

In mammals, the circadian timing system influences most physiological activities, including body temperature, cardiovascular activity, sleep–wake cycles, acuity of the sensory system, renal plasma flow, intestinal peristaltics, and many functions of the endocrine system (Schibler *et al*. 2003). All these rhythmic functions influenced by the SCN, which receives photic information from the melanopsin-expressing ganglion cells of the retina. The discovery of several rhythmically expressed mammalian clock and clock-controlled genes has made it possible to monitor circadian rhythms in gene expression in any cell type. Such studies have shown that most body cells-and even cells cultured *in vitro*-harbour circadian oscillators with a similar molecular makeup as those that operate in SCN neurons.

Molecular circadian clocks govern the daily expression of thousands of tissue-specific genes (Valekunja *et al*. 2013). Disharmony between these circadian clocks and environmental cues is referred to as circadian disruption. Circadian disruption may lead to the various long-term diseases, including cancer (Stojkovic *et al*. 2014; Arellanes-licea *et al*. 2014; Reddy *et al.* 2014). Disruption of circadian rhythms may also lead to epigenetic modifications, which may alter cell proliferation and therefore result in oncogenesis and cancer (Haus *et al*. 2013). Epigenetic changes can be the result of several environmental factors, including repeated circadian disruption due to long-term shift work. Studies on shift workers have demonstrated changes in the DNA methylation of their genes (Haus *et al*. 2013).Circadian disruptions are also result of industrialization and the development of societies and consequent changes in lifestyle (Shanmugam *et al*. 2013). It has been reported that approximately 20% of workers have shift work schedules worldwide. The International Agency for Research on Cancer (IARC) reported that shift work may be a carcinogenic factor in humans (Kubo *et al*. 2013). In addition, studies of breast cancer in women with shift work schedules have provided more evidence for the carcinogenic effects of circadian disruption (Gapstur *et al*. 2014).

Several environmental factors, such as night-shift work, exposure to artificial light, irregular diet, and electromagnetic (EM) waves, which affect biological processes mostly by altering melatonin rhythms, result in circadian disruption (Shanmugam *et al*. 2013). Because light is the most potent synchronizer of circadian rhythms to the external environment, night-shift work and exposure to artificial light are the strongest disruptive factors of circadian rhythms (Fonken *et al.* 2014). Furthermore, several circadian genes, such as the *PER* (Period) family genes, circadian locomotor output cycles kaput (*CLOCK*) and cryptochrome circadian 2 (*CRY2*), can be affected by insufficient sleep (Möller *et al*. 2013).

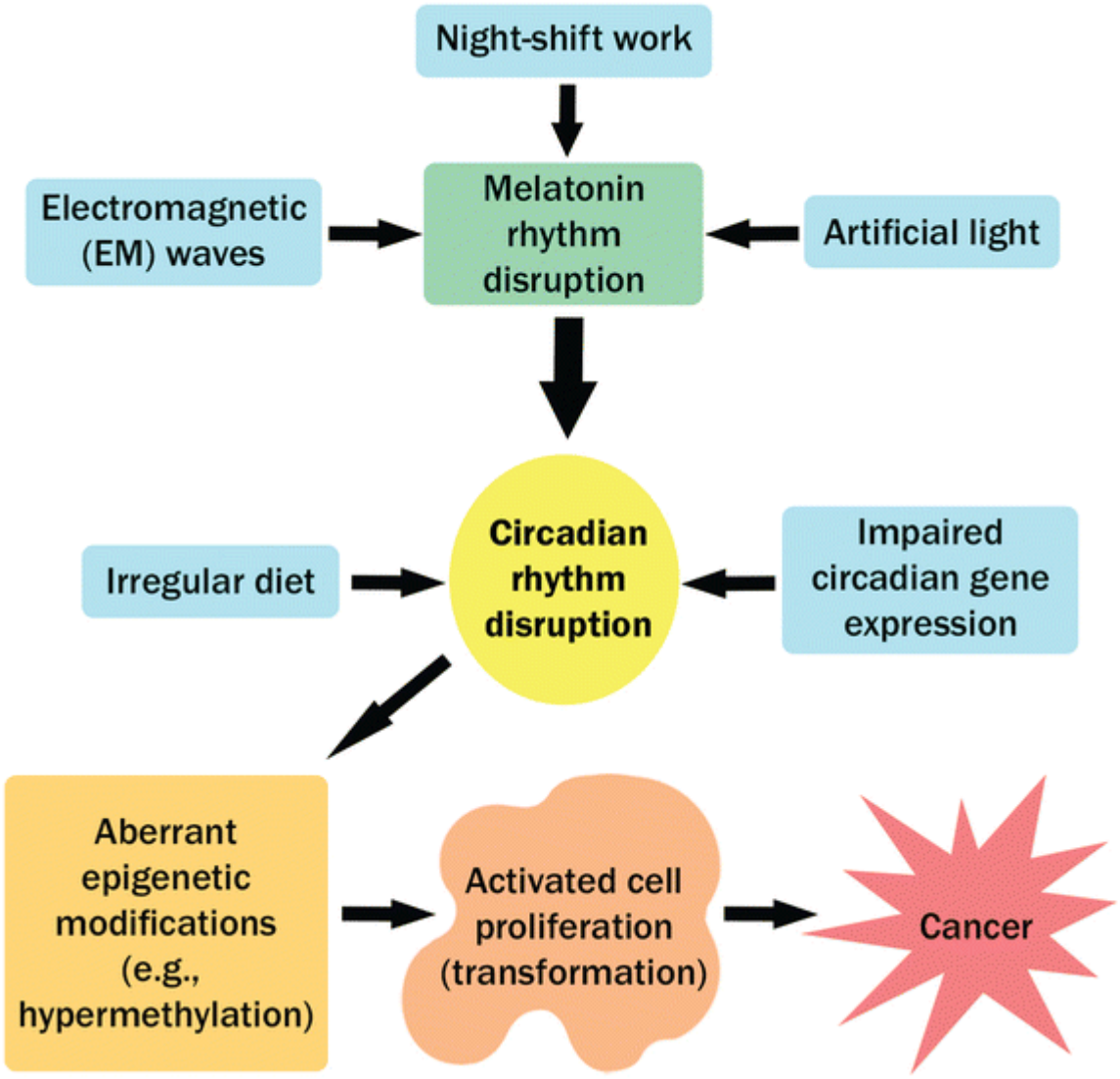

Figure1:Different circadian disruptive factors that lead to cancer.

Along with the core-clock genes, the synchronization of circadian rhythms results due to various clock-controlled genes, including several cell cycle genes (Rana *et al*. 2014). The clock machinery and cell cycle are controlled by similar mechanisms that include feedback loops. The clock machinery has functional interactions with cell cycle regulators, so that changes in clock function result in uncontrolled cell cycle progression and cell proliferation (Soták *et al*. 2014). Due to associations between the circadian clock and cell metabolism, circadian disruption results in abnormal cell metabolism. All of these abnormalities are important factors in the process of carcinogenesis and can result in multi-tumorigenesis

(Sahar *et al.* 2009; Li *et al*. 2013; Masri *et al.* 2013). Disruption of the expression of clock genes has also been found in cancer patients (Mazzoccoli *et al*. 2014). The core clock genes*PER1* and *PER2* are known tumor suppressor genes, and their knockdown results in the doubling of tumor number and cancer growth; in contrast, over expression of these genes decreases tumor number and cancer growth (Hrushesky *et al*. 2009).

Along with epigenetic aberrations (Mathew *et al*. 2014), exposure to night light reduces melatonin levels and may lead in increased estrogen production and altered estrogen receptor function, which may cause increased breast cancer risk (Stevens 2005). It has been demonstrated that the expression levels of the human *CRY1*, *CRY2*, *PER1*, *PER2*,*PER3*, and *BMAL1* genes were down-regulated in both the chronic phase and blast crisis in chronic myeloid leukemia (CML) (Yang *et al*. 2006). Aberrant hypermethylation of the promoters of certain genes is an important hallmark of cancer cells. Elevated risk of fatal ovarian cancer has an important association with a rotating work schedule, but it has no significant association with sleep duration or insomnia. It has been reported that disruption of circadian rhythms in shift workers is associated with an increased incidence of colorectal neoplastic disease (Mazzoccoli *et al*. 2014). Short sleep duration, insomnia, and shift work schedules are some of the factors that disrupt circadian rhythms. The Cancer Prevention Study–II group performed a prospective study on men who experienced these circadian disruptive factors (Gapstur *et al.* 2014). They suggested that certain aspects of sleep disruption may increase the risk of prostate cancer (Sigurdardottir *et al.* 2013). Disruption of circadian rhythms in lung function has been observed in patients with obstructive lung disease. Another study on the epigenetic basis of non–small cell lung cancer (NSCLC) used microarray analysis to focus on tumor suppressor genes silenced by DNA methylation and histone deacetylation. These results indicated that circadian disruption plays an important role in lung tumorigenesis (Gery *et al*. 2007). Gapstur *et al*. (2014) investigated the expression of eight circadian clock genes

including *PER1*, *PER2*, *PER3*,*CRY1*, *CRY2*, *BMAL1*, *CLOCK*, and *CKIε* in a study on cancerous and non-cancerous tissues from 29 gastric cancer patients. They reported that *PER2* was notably up-regulated in cancer tissues compared with non-cancerous tissues. In addition, up-regulation of *CRY1* expression was markedly associated with the advancement of clinical stages of gastric cancer. They suggested that disruption of circadian rhythms may be associated with the development of gastric cancer (Gapstur *et al*. 2014).

Circadian clock is also connected to neurological and mental disorders. Circadian rhythms play an obvious role in controlling sleep cycles and have been linked to seasonal affective disorder (SAD), a mood disorder with symptoms of depression only during a certain time of the year, depression and neurodegenerative diseases (Albrecht 2013; Videnovic *et al*. 2014; Waddington *et al*. 2007). Another prominent example is the familial advanced sleep phase syndrome, an inheritable disease caused by a point mutation in the *PER2* gene. Together, these examples show the strong influence of circadian biology on health and diseases. Much less is known about the impact of circadian rhythm disruption on gastrointestinal health in humans, despite the fact that there is significant circadian regulation of digestive system activity (Hoogerwerf 2009;Hoogerwerf*et al*. 2007, 2008; Polidarová *et al*. 2011; Scheving 2000;Scheving and Russell 2007). Disruption of gastrointestinal barrier function (by disease or environmental factors such as alcohol) can increase intestinal permeability ("gut leakiness"), thus enabling the translocation of bacterial products such as endotoxin, from the intestine into the circulation, triggering inflammatory cascades that can promote or exacerbate inflammatory-based diseases (Farhadi *et al*. 2003; Turner, 2009; Wang *et al.*2012).

The medical importance of research on circadian rhythms is exemplified by applications in the treatment of hypertension (Cornélissen *et al*. 1991; Hermida *et al.* 2003,

2004), cancer (Halberg *et al*. 2003; Hrushesky 1985; Lévi *et al*. 1997), rheumatoid arthritis (Günther *et al*. 1980), asthma (Pincus *et al.* 1995), the malaise associated with shift work (Boivin and James 2002; Crowley *et al.* 2003), sleep disorders (Czeisler *et al.* 1981), and numerous other conditions. It has long been observed that cancers have altered metabolism (Warburg 1956; Vander Heiden *et al*. 2011; Stine and Dang 2013), and that many cancers may have disrupted circadian rhythm (Levi *et al.* 2008); however, the significance and mechanism of the circadian dysrhythmia in cancer are poorly understood.

The present thesis

This thesis includes investigations on the Mizo ethnic population. In particular, the emphasis in this study is placed on studying the status of biological rhythms in the patient suffering from upper intestinal cancer and their comparison with the healthy subjects. Various studies are summarized in following three sections.

Section 1:To study cardiovascular rhythms in cancer patients

This section deals with daily changes in cardiovascular parameters (systolic blood pressure, diastolic blood pressure, mean arterial pressure, pulse and double product) recorded in patients suffering from upper intestinal cancer and their comparison with the healthy subjects.

Section 2:To study activity rest pattern in cancer patients

This section deals with daily changes in activity rest patterns (daily activity count, High Proportional Integrative measures (HPIM) and Proportional Integrative measures performances) recorded in patients suffering from upper intestinal cancer and their comparison with the healthy subjects.

Section 3:To study the sleep quality in cancer patients

This section deals with changes associated with the sleep quality and performance in patients suffering from upper intestinal cancer and their comparison with the healthy subjects using Pittsburgh Sleep Quality Index (PSQI) and The Epworth Sleepiness Scale (ESS).

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SECTION 1: TO STUDY CARDIOVASCULAR RHYTHMS IN CANCER PATIENTS

1. Introduction

Daily blood pressure variation is under circadian regulation, governed by the cyclic variation of the sympathetic nervous system. The rise in blood pressure is steep and abrupt during the first few hours of awakening and coincides with the peak incidence of many acute and life-threatening cardiovascular and cerebrovascular events, for example myocardial infarction, stroke, sudden death and ventricular arrhythmias. Blood pressure variability is one of the known risk factors for different types of cardiovascular diseases. Several physiological, behavioral and ethnic factors are known to modulate blood pressure (BP). Peripheral clocks also exist in cardiovascular tissues as in other organs (Maemura *et al*. 2007) and there is rhythmic expression of clock genes in the heart, aorta, and kidney (Maemura *et al.* 2000; Nonaka *et al*. 2001). Microarray analysis revealed that, 8—10% of the genes exhibit circadian expression in the heart (Storch *et al*. 2002). The day-night variations of blood pressure and heart rate coincide with diurnal variability in many cardiovascular diseases (CVDs) such as cardiac arrhythmias, atherosclerosis and sudden death (Portaluppi *et al*. 2012; Yang *et al*. 2013). The timing of sudden cardiac death displayed circadian variability, prominent in the early morning. Both atrial and ventricular arrhythmias appear to exhibit circadian patterning as well, with a higher frequency during the day than at night (Portaluppi *et al*. 2012).

Circadian rhythms in timing of onset and tolerance to myocardial infarction (MI) have been well documented. The occurrence of MI is more frequent in the morning than at night (Culic 2014). During early morning hours, the increased in systolic blood pressure and heart rate leads in an increased energy and oxygen demand by the heart, however the vascular tone

of the coronary artery rises in the morning, resulting in a decreased coronary blood flow and oxygen supply. This mismatch between supply and demand elicits the high frequency of onset of MI. Disruption of circadian rhythm like shiftwork and jetlag has been well established to be a risk factor for many CVDs, including MI (Knutsson *et al*. 1999). Even 1 h shift can significantly increase the chances of MI occurring (Janszky and Ljung 2008). Clock genes may also exert non-clock roles in the cardiovascular system. For example, activation of an adenosine receptor *ADORA2B* acts via *PER2*, to induce an energy utilization switch from fatty acid to glucose in cardiomyocytes, which promotes glycolysis and protects against cardiac ischemic injury (Eckle *et al*. 2012; Yang and Fitzgerald 2012).

Numerous studies from animal models proved the adverse effects of circadian disruption in CVDs. Mouse hearts in rhythm-disruptive environments are prone to malfunctions with altered clock gene cycling and reduced contractility (Martino *et al*. 2007). Deletion or mutation of Clock in mice dampened cardiovascular rhythms accompanied by dilated cardio-myopathy (Lefta *et al*. 2012), arterial stiffness (Anea *et al.* 2010), or endothelial dysfunction (Viswambharan *et al*. 2007). Further CLOCK mutant mice exhibited the impaired cholesterol metabolism and increased development of atherosclerosis. CVDs also affect clock gene expression. In salt sensitive rats, high salt diet induces cardiac hypertrophy, associated with attenuated rhythmic expression of clock genes (Mohri *et al.* 2003). In a type 2 diabetic rat model, clock genes in cardiac tissue exhibit a 3 h phase delay, suggesting a loss of normal synchronization in diabetic hearts (Young *et al.* 2002). Aortic constriction overloads the pressure, results in decrease of the amplitude of circadian expression of clock genes in the rat heart (Young *et al*. 2001; Durgan *et al*. 2005).

In humans, along with circadian rhythms, circannual, circaseptan and circasemiseptan rhythms have also been reported for blood pressure and heart rate (Rawson *et al*. 2000;

Halhuber *et al*. 2002). The pattern of blood pressure variability (BPV) alters in case of depression (Rawson *et al*. 2000). Variability in various cardiovascular parameters has been studied in healthy subjects with reference to age, gender and ethnicity (Harshfield *et al*. 1989; Suzuki *et al.* 1993; Driziene *et al*. 2008) and increased mesor, decreased amplitude and advancement in acrophase of systolic and diastolic blood pressure with advancing age has been reported (Suzuki *et al*. 1993). Similarly, age and gender modulate the heart rate variability of healthy subjects (Umetani *et al*. 1998) and HRV was found to be decreased with the advancement of age (O'Brien *et al*. 1986; Shannon *et al.* 1987; Ori *et al*. 1992; Umetani *et al.* 1998). Therefore, it is recommended that the circadian rhythm of BP should always be examined with reference to sex-, age- and race-matched reference values (Harshfield *et al*. 1989; Suzuki *et al*. 1993; Driziene *et al*. 2008).

The, non-invasive ambulatory blood pressure monitoring (ABPM) has been utilised to explore the impact of blood pressure variability on cardiovascular morbidity and mortality (Mancia and Parati 2000, Yadav *et al*. 2014). In most studies rhythms/clock genes studies have been done in reference to colorectal/rectal cancer however, nothing is known how the upper intestinal cancer (oesophageal cancer) is influencing biological rhythms or vice versa. In present study, we recorded the cardiovascular rhythms in the patients suffering with the upper intestinal cancer and compared it with the normal healthy subjects.

2. Materials and Methods

This study was performed between the months of September 2016 to October 2017. The patients for upper intestinal cancer (esophageal cancer) were selected from Nazareth Cancer Hospital, Chatlang and Mizoram State Cancer Institute, Zemabawk, Aizawl, Mizoram, India while the healthy volunteers were included from the city of Aizawl. All subjects (patients and healthy subjects) with no signs of any cardiovascular diseases or having sleep related problems were included in the study.

Prior to begin the study, all volunteers were informed about its background and purpose and written consents were obtained. However, the volunteers were allowed to withdraw their participation from the study at any point of time. Those volunteers that did not complete the whole study data are not included for analysis. Individual confidentiality was maintained throughout the study. During the study period, the subjects were allowed to do their daily routine work. Permission for this study was obtained from the Institutional Human Ethics Committee and authorities of the above two mentioned hospitals.

A standard oscillometric ABPM device (A & D Company Limited, Tokyo, Japan, TM-2430) was used to record the cardiovascular parameters:

Systolic blood pressure (SBP): When heart beats it contracts and pushes blood through the arteries to the rest of the body, this force creates pressure on the arteries and is called systolic blood pressure.

Diastolic blood pressure (DBP): The diastolic blood pressure indicates the pressure in the arteries when the heart rests between the beats.

Mean arterial pressure (MAP): mean arterial pressure is defined as the average pressure in a person's arteries during one cardiac cycle.

Pulse: A pulse represents the tactile arterial palpation of the heartbeat.

Double product (DP): The double product (DP), consisting of the systolic blood pressure multiplied by the pulse rate, is an index of myocardial oxygen consumption.

The cuff of the ABPM device was worn on the left arm and programmed to record the parameters at every 1-h interval in a 24-h day for the two consecutive days. Data was transferred from the device to the computer and analyzed using Doctor Pro3 Software manufactured by A and D Company Limited.

3. Statistical analysis

Data represented as mean ± SE. One way analysis of variance (One way ANOVA) revealed the effect of time within the same group and two-way analysis of variance (two way ANOVA) was used to determine the effect of health conditions (patients vs healthy subjects) along with time variable. Significance was taken at $p < 0.05$. Post hoc tests were used to study the effects between the two parameters.

4. Results

Figure 1 shows the various cardiovascular parameters studied in the cancer patients and their comparison with the healthy subjects. Both healthy subjects as well as cancer patients showed daily variations in the systolic blood pressure (Control: $F_{23,552} = 2.186$, $P < 0.0001$; patients: ($F_{23,782} = 14.12$, $P < 0.0001$; Figure1a), diastolic blood pressure (Control: $F_{23,552} = 2.727$, $P < 0.0001$; patients: $F_{23,782} = 5.314$, $P < 0.0001$; Fgure1b), MAP (Control: $F_{23,552}= 1.443$, $P < 0.0001$; patients: $F_{23,782} = 6.557$, $P < 0.0001$; Figure1c), Pulse (Control: $F_{23,552}= 3.140$, $P < 0.0001$; patients: $F_{23,782} = 1.912$, $P < 0.0001$; Figure1d) and in the double product (Control: $F_{23,552} = 15.45$, $P< 0.0001$; patients: $F_{23,782} = 17.04$, $P < 0.0001$; Figure1e). In general, both in healthy subjects as well as in patients, higher systolic blood pressure during the day time and lower systolic pressure during the night time was recorded ($p < 0.05$, Newman–Keuls test; Figure1a). In healthy subjects, maximum systolic blood pressure was recorded around 13 'O' Clock (Mean ± SE: 139± 4.3 mm Hg) while in patients it was slightly advanced and around 11 'O' clock (Mean ± SE: 127± 3.8 mm Hg). Similarly, in healthy subjects, minimum systolic pressure was recorded at 2 'O' clock (Mean ± SE: 112± 1.6 mm Hg) while in patients it was advanced and around 1 'O' clock (Mean ± SE: 103± 2.7 mm Hg). Comparison of various cardiovascular parameters of cancer patients with healthy subjects suggest there is an effect of health conditions; systolic blood pressure ($F_{1,888} = 137.0$, $P < 0.0001$), time ($F_{23,888} = 7.457$, $P < 0. 0001$) and interaction of condition X time ($F_{23,888} = 2.005$, $P = 0.0035$; Two-way RM ANOVA; Figure1a); diastolic blood pressure ($F_{1,888} = 394.3$, $P < 0.0001$), time ($F_{23,888} = 5.188$, $P < 0. 0001$) and interaction of condition X time ($F_{23,888} = 7.594$, $P = 0.0035$; Two-way RM ANOVA; Figure1b); MAP ($F_{1,888} = 286.7$, $P < 0.0001$), time ($F_{23,888} = 7.457$, $P < 0. 0001$) and interaction of condition X time ($F_{23,888} = 2.005$, $P = 0.0035$; Two-way RM ANOVA; Figure1c); pulse($F_{1,888} = 215.4$, $P < 0.0001$),

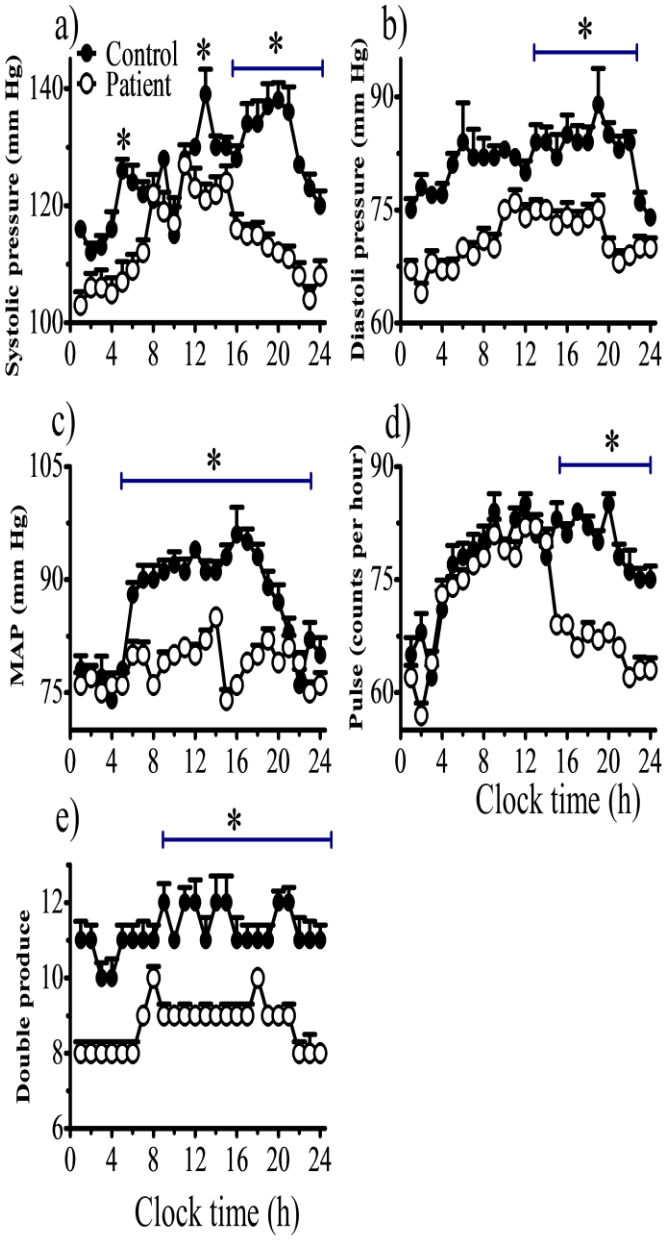

Figure 1. Various cardiovascular parameters studied in the upper intestinal cancer patients represented by hallow circle and their comparison with healthy subjects represented by solid circle. Data is represented as mean ± SE. Systolic blood pressure (a), diastolic blood pressure (b), mean arterial pressure (c), pulse (d), and double product (e). Asterisk (*) indicates a significant difference between healthy subjects and cancer patients ($p < 0.05$, Post hoc test).

time ($F_{23,888}$ = 25.74, P < 0. 0001) and interaction of condition X time ($F_{23,888}$ = 28.287, P = 0.0035; Two-way RM ANOVA; Figure1d); double product ($F_{1,888}$ = 486.3, P < 0.0001), time ($F_{23,888}$ = 5.310, P < 0. 0001) and interaction of condition X time ($F_{23,888}$ = 2.009, P = 0.0045; Two-way RM ANOVA; Figure1e). In general, the various cardiovascular parameters studied were suppressed in patients in comparison to healthy subjects. In comparison to healthy subjects, in patients the systolic blood pressure starts falling down much earlier and minimum systolic blood pressure was attained 1hour advance in comparison to healthy subjects ($p < 0.05$, Bonferroni test).

5. Discussion

Present study shows that there is a daily variation in the different cardiovascular parameters studied and hence in the blood pressure of both healthy subjects as well as cancer patients are under circadian regulation. Our findings are in consistent with the previous reports showing the daily variations in the blood pressure (Redon 2004) of healthy subjects. We observed higher blood pressure during the day time and lower blood pressure during the night time (Figure1). In humans, higher Blood pressure during the daytime (between 10.00 a.m. and 6.00 p.m.) (Millar-Craig *et al.* 1978) and lower blood pressure at night time, with a characteristic dip in blood pressure between midnight and 3.00 a.m. (Redon 2004) has been reported. In our study in case of healthy subjects we observed rise in systolic and diastolic blood pressure, pulse, MAP and DP between 6.00 a.m. to 8.00 p.m. (figure 1) while in case of patients we observed similar pattern but with less amplitude (Figire1). In previous studies, slow and steady rise in blood pressure was recorded between 3.00 a.m. and 6.00 a.m. (Millar-Craig *et al.* 1978), we observed similar phenomenon in our study. However, in comparison to healthy subjects, cancer patients showed suppression of all cardiovascular parameters. Effects of disease conditions on the cardiovascular rhythms have been observed.

Circadian rhythm in blood pressure has been studied in many normotensive subjects and patients with primary hypertension (Kohno *et al*. 1998;Rawson *et al*. 2000; Hermida *et al*. 2001; Halberg *et al*. 2004). In general, the circadian rhythm of blood pressure in individuals with essential hypertension in comparison to normotensive subjects, the blood pressures are reported consistently elevated in comparison with normal values (Mancia *et al*. 1997) and the amplitude of variation between peak and trough values may be altered. In majority of humans, including hypertension subjects, blood pressure typically drops 10–20% during dark hours (Millar-Craig *et al.* 1978). However, this usual night-time dip in blood pressure has not been observed in some individuals. Studies using ABPM have shown that, as

the age progresses (over 65 years of age), blood pressure does not fall during the night (Harshfield *et al.* 1990; Staessen *et al*. 1997; White 2000). Diabetes conditions and chronic renal failure also lead the circadian variability in the blood pressure parameters (Lurbe *et al.* 2001; Redon 1998). In a clinical condition, when decline in nocturnal blood pressure is less than 10% of the daytime blood pressure, people are termed as 'non-dippers' (O'Brien *et al*. 1988; Pickering 1990). These individuals have a poorer cardiovascular performances and more severe target-organ damage than patients whose blood pressure does fall at night (Verdecchia *et al.* 1994; Verdecchia 2000). In case of type 1 diabetes, a non-dipping blood pressure pattern has been linked with the higher risk of developing early diabetic nephropathy (Lurbe *et al.* 2002).

The characteristics of circadian rhythm of blood pressure parameters are altered in diseased persons. Disappearance of the circadian blood pressure rhythm in patients with secondary ornon-dipper type hypertension has been reported (O'Brien *et al*. 1988). However, in our study patients suffering with upper intestinal cancer did not lose the daily rhythms in different cardiovascular parameters; but there was a significantly reduction in the amplitude of the different cardiovascular paramters we studied (Figure 1).The desynchronized rhythm in BP is associated with increased risk of cardiovascular complications (Profant and Dimsdale 1999; Parati *et al*. 2003; Pistrosch *et al*. 2007) and that cardiovascular risk is more in diabetic patients and fasting hyperglycemia, associated with abnormal diurnal BP variation. Association of BP and metabolic syndrome in untreated hypertension has also been reported (Hermida *et al.* 2009). It has been reported that non-dippers are at higher risk than dippers (Ohkubo *et al*.2002). Further, it has also been proposed that risk of cardiovascular complications is on the rise among young individuals of this region.

Blood pressure and heart rate are influenced by both endogenous and exogenous factors (Waterhouse *et al.* 2007). The present results showed that circadian rhythm parameters of pulse exhibited daily variations with significant difference as function of time between healthy subjects and cancer patients. The study conducted on young normotensive subjects showed that circadian rhythm parameters of heart rate did not exhibit any significant difference as function of gender and nocturnal dipping pattern except for mesor between males and females (Vaidya *et al*. 2012). In our study, we did not take separate consideration of male and female as a relatively less number of sample size and therefore we do not speculate the results in terms of gender. However, we clearly see effect of health conditions on the daily variation of the pulse and mean arterial pressure where in comparison to the healthy subjects, amplitude is reduced in cancer patients. In one of the earlier reports it has been documented that HRV is affected to a lesser extent by the gender (Jensen-Urstad *et al*. 1997). However, Umetani *et al*. (1998) reported that heart rate variability is influenced by the gender, but it is age dependent. Similarly, in the hyperthyroid conditions heart rate and pulse pressure were significantly higher in the group as compared to the control group (Kohno *et al.* 1998).

There are some limitations of the present study due to sample size and we did not consider the effect of gender and age separately. Further, for cancer patients we have not considered chemotherapy as a separate marker for the effect on the cardiovascular rhythms. These are the issues we may address in our future research problems. However, on the basis of our findings we can suggest that there is a effect of health conditions (upper intestinal cancer) on the expression of cardiovascular rhythms and in general the various parameters of cardiovascular systems are reduced in terms of amplitude.

6. References

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SECTION 2: TO STUDY ACTIVITY REST PATTERN IN CANCER PATIENTS

1. Introduction

Clinically, wrist watch based actigraphy is used to determine the daily rest-activity rhythm of patients (Chevalier *et al.* 2003; Levin *et al*. 2005; Mormont and Waterhouse 2002). Initially, medically actigraphy was used to attempting to evaluate psychologic disorders in the pediatric population and it was based purely on mechanical sensors first conceived in the 1950s (see Tryon *et al.* 1991). Later on, as the advancement of technologies, the development of piezoelectric sensors, lithium batteries, and digital data storage has enhanced accuracy, reliability, and storage capacity. As of now, the devices can collect, long-term data regarding of a subject's daily activity pattern. It is rapidly developing into a significant asset for assessing circadian as well as sleep related complications and in their clinical applications. The field of actigraphy with its increasing usefulness with advancement of devices, is used for measuring body movement with high precision and frequency, and current devices are able to record/store information for months. With the help of the advanced software packages and to the development of automatic scoring algorithms can better use for issues related with sleep vs wakefulness. Current devices are advantageous due to its small size and light weight, therefore making the devices unobtrusive and convenient for subjects to wear it. Due to the collection of data as represented by body movement over time, the actigraph presents a picture of daily sleep-wake cycles, which is useful in the diagnosis and evaluation of several clinical sleep disorders and treatment outcomes. Further, growing research literature support the use of actigraphy for clinical application, particularly in the evaluation of circadian rhythm disorders, insomnia, hypersomnia, and obstructive sleep apnea (Morgenthaler *et al*. 2007).

Actigraphy is a non-invasive method of monitoring individual rest/activity cycles. In general, it consists of a small unit, called an actimetry sensor (Pigot *et al*. 2003),can be worn to measure gross motor activity. In general, it is a wrist-watch-like, worn on the wrist of non-dominating arm. The actigraph unit undergoes are continually recorded and some units also measure light exposure. The data can be later downloaded and read to a computer and analysed offline with appropriate software systems; in some brands of sensors the data can be transmitted and analysed in real time. Actigraphy, ultimately measures the wrist (therefore considered as a general body movement) movements during sleep/activity, which proved to be an indication of wake state (Ancoli-Israel *et al*. 2003; Pollak *et al*. 2001). Up to a certain extent, actigraphy would often be reflected in their respiratory effort signal as body motion artefacts during measurement. It is a simple method to study sleep related disorders and the rest-activity cycle (Ancoli-Israel *et al*. 2003). The various parameters use for actigraphy have been regularly updated (Littner*et al*. 2002; Morgenthaler *et al*. 2007). It can also be useful in recurrent mood disorder. The attention deficit disorder shows high levels of motor activity during the day and the night, and use of methylphenidate shortens their total sleep time, but improves sleep fragmentation (Boonstra *et al*. 2007).

Actigraphy can also be a useful tool for evaluating insomnia, as insomnia have a greater propensity for misperceiving the sleep time of the individuals than subjects without insomnia and overall tend to significantly underestimate sleep time (Means *et al.* 2003).It can also be used to assess treatment effects in circadian rhythm disorders. A number of studies have demonstrated the successful use of actigraphy to follow the treatment of phase advancement, (Ondzé *et al*. 2001) delay,(Nagtegaal *et al*. 1998) jet lag, (Beaumont *et al*. 2004; Burgess *et al*. 2003) and shift work sleep disorder (Kubo *et al*. 2009; Bjorvatn *et al*. 2007).Wrist actigraphy is a valuable measure of sleep activity and circadian rhythms. Although not used for diagnosis of clinical sleep disorders, in general, actigraphy is used as a

research tool to study various populations. Sleep–wake cycles and circadian rhythms, especially those of rest and activity, are closely linked (Palesh *et al*. 2012; Lavie 2001). An alteration of the circadian rest–activity rhythm has been shown to be an independent negative prognostic factor in survival in patients with advanced colorectal cancer (Mormont *et al*. 2000; Innominato *et al*. 2009; Innominato *et al*. 2012; Levie *et al*. 2014).

The circadian activity-rest rhythm has been used to investigate as a potential marker of the internal circadian time organization (Chevalier *et al*. 2003; Mormont and Waterhouse, 2002). The circadian rest-activity rhythm dampens in patients suffering from head and neck cancer (Pati *et al*. 2006). In general, rest–activity rhythm pattern changes with age; advance in phase, shortening of the period, and desynchronization are common among elderly human subjects (Buysse *et al*. 2005; Czeisler *et al*. 1992; Kripke *et al*. 2005). Elderly people have tendency to go to bed early and get up early (Rodriguez *et al.* 2015). Further, some elder people also experience a decrease in amplitude of rhythm. The rest–activity rhythm mediates the relationship between physical activity and quality of life in elderly cancer survivors (Su *et al*. 2015). Both the genetic and environmental factors can negatively affect the rest–activity rhythm, and age can accelerate pathological changes (Yu and Weaver, 2011). In cancer patients, the rest-activity rhythm has been reported to be altered even before the administration of chemotherapy (Innominato *et al*. 2009; Mormont *et al*. 2000; Ancoli-Israel *et al*. 2006; Berger *et al*. 2007). Previous studies have shown that chemotherapy induces rapid-onset and sustained alterations in the rest-activity rhythm in most cancer patients (Ortiz-Tudela *et al*. 2014; Ancoli-Israel *et al*. 2006; Berger *et al*. 2007; Savard *et al*. 2009).

However, in most of the studies in cancer patients have been done on the patients suffering with breast or rectal cancer, less is known about the patients suffering with the upper intestinal cancer. Therefore, we addressed the status of activity-rest pattern in the

patient suffering with upper intestinal cancer.

2. Materials and Methods

The actiwatch (Motionlogger Watch, version:1.60.0.1CamNtech Ltd. United Kingdom) was used to record the activity rest pattern in patients and healthy subjects. Motionlogger is a wrist worn device that measures activity-rest or sleep-wake rhythm. The activity was assessed continuously and recorded in 1minute epochs throughout 24-hr day for 2 consecutive days of the study period. The subjects were instructed not to remove the device on data collection days except when they go to bath. The Motionlogger watch helps to provide accurate and objective activity, sleep, wake, and light-exposure data. Data was analyzed using Action 4 software. Action4 is a multi-channel software system designed to process data retrieved from all of Ambulatory Monitoring's physiologic recording devices. Initialization and data retrieval for each of these devices is performed with software. The Action4 software is specifically for display and analysis of these data.

Actigraph Sampling Modes: Various actigraphy sampling modes includes:

Zero Crossing(ZC): This mode measures the frequency of movements and is necessary to access the validated sleep estimation algorithms available in the analysis software packages of Action W and Action 4. It also represents the total movement in terms of counts.

High proportional integrative measures (HPIM): High Proportional Integrative measures (HPIM) reflects the activity performances.

Proportional Integrative measures (PIM): Proportional Integrative measures (PIM) reflects the intensity of movement. This mode does a high-resolution integration of the area under the rectified (absolute value) acceleration signal. This mode is valuable for discriminating different intensities of motion (sitting, walking, running).

Life Measures: This is a proprietary mode of operation which picks up the body's micro-vibration and rarely goes to zero when worn on the human body. This channel can be used to help delineate off-wrist intervals.

Events: Pressing and releasing the upper-left button, marked Event, places a mark in memory which can be viewed in any analysis program. The meaning of the event marker is up to the investigator. The character "E" is presented on the display as feedback to the wearer that an event has been marked.

User Entry: This feature allows entry of a value of 0-10 for marking distinct events by a number or for performing a subjective rating scale. The scale presented begins at 5 and the user can press and release the UP and/or DOWN buttons to any other available digit. Pressing and releasing the MODE button again accepts the displayed value and stores it, time-stamped, in memory. If the watch is allowed to time-out (30-sec) without accepting a value, then no result is recorded.

Delta Temperature: While this channel is not offered as a diagnostic measure, a rapid decrease or increase temperature within the device case is associated with Watch removal or application, respectively in most individuals and climate conditions. This channel can be used to enchance off-wrist determinations especially when used in conjunction with the Life Measure channel.

Ambient Light: A light sensor mounted at the lower center of the Watch face, below the glass is normally calibrated in between 0 and 2700 Lux. This channel is intended for the purpose of making educated decisions about time in bed based on "light out" and "light-on" indications around the habitual bedtime of the subject. The sensor can also give an approximation of when the subject is in the presence of low, moderate or bright light. Also, a steady light level with little or no variance is another indicator that the Motionlogger Watch

is “off-wrist” and has been placed in some fixed position with respect to the ambient light source.

Diagnostic: This mode invokes a recording of individual channels of ZCM type data from various axes of the accelerometer as well as battery voltage readings and a multitude of system specific events.

3. Statistical analysis

Data represented as mean ± SE. One way analysis of variance (One way ANOVA) revealed the effect of time within the same group and two-way analysis of variance (two way ANOVA) was used to determine the effect of health conditions (patients vs healthy subjects) along with time variable. Significance was taken at $p < 0.05$. Post hoc tests were used to study the effects between the two parameters.

4. Results

Figure 2 shows the various actigraphy parameters studied in the cancer patients and their comparison with the healthy subjects. Both healthy subjects as well as cancer patients showed daily variations in the daily activity count (Control: $F_{23,552}$ = 21.20, P < 0.0001; patients: $F_{23,782}$ = 24.32, P < 0.0001; Figure 2a), HPIM (Control: $F_{23,552}$ = 14.64, P < 0.0001; patients: ($F_{23,782}$ = 14.32, P < 0.0001; Figure 2b) and PIM (Control: $F_{23,552}$ = 14.69, P < 0.0001; patients: ($F_{23,782}$ = 13.17, P < 0.0001; Figure 2c). In general, both in healthy subjects as well as in patients, higher activity during the day time and lower activity during the night time was recorded (p < 0.05, Newman–Keuls test; Figure 2a). In healthy subjects, maximum activity was recorded around 13:00 h Clock (Mean ± SE: 6035± 1287counts/h; Figure 2a) while in patients it was slightly advanced and around 16:00h (Mean ± SE: 5074± 1160counts/h; Figure 2a). However, both in healthy subjects as well as in patients, minimum activity was recorded at 04:00 h (healthy subject: Mean ± SE: 203± 66 counts/h Patients: Mean ± SE: 103± 2.7 counts/h; Figure 2a). Comparison of daily activity of healthy subjects with patients suggest there is only effect of time (activity count: $F_{23,1152}$ = 17.23, P < 0. 0001; HPIM: $F_{23,1152}$ = 16.29, P < 0. 0001; PIM: $F_{23,1152}$ = 16.30, P < 0. 0001) but not of health conditions ($F_{1,1152}$ = 1.118, P = 0.2365; HPIM: $F_{23,1152}$ = 1.231, P = 0.2432; PIM: $F_{23,1152}$ = 1.532, P =1850) or interaction of health condition X time ($F_{23,1152}$ = 1.281, P = 0. 2425; HPIM: $F_{23,1152}$ = 1.253, P = 0.1691; PIM: $F_{23,1152}$ = 1.286, P =0.1650;Two-way RM ANOVA). In general, the various actigraphy parameters studied in the present study were similar in both patients as well as in the healthy subjects.

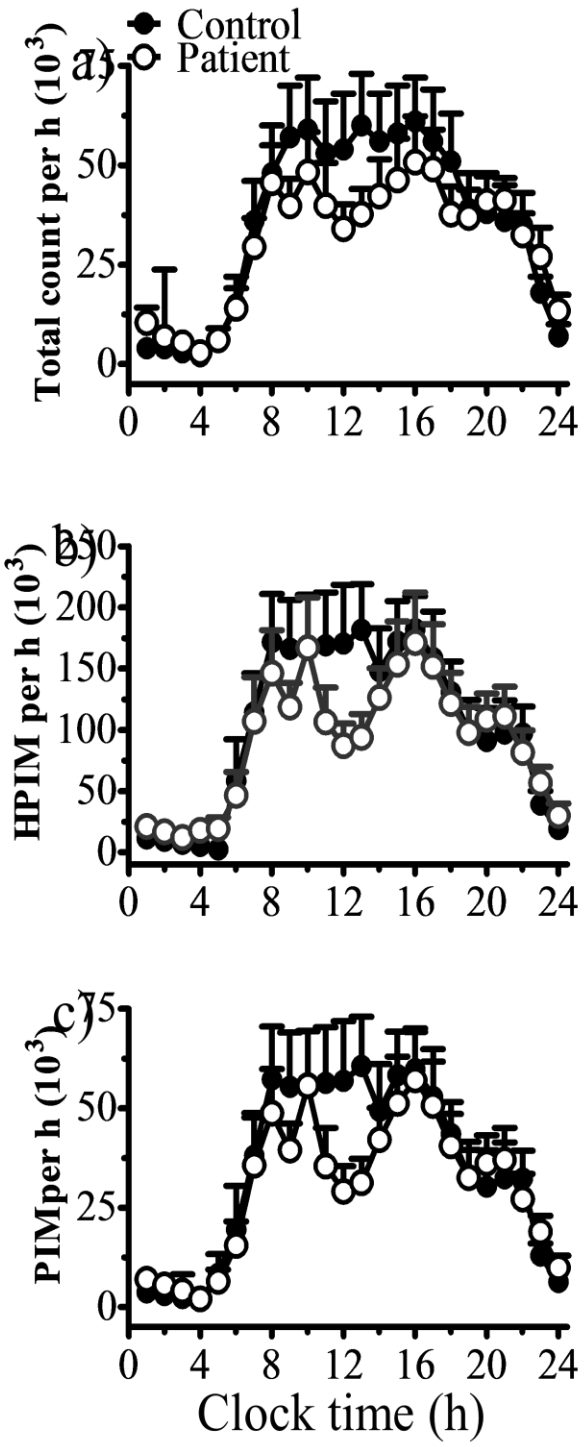

Figure 2. Various activity rest parameters studied in the upper intestinal cancer patients represented by hallow circle and their comparison with healthy subjects represented by solid circle. Data is represented as mean ± SE. Total activity count (a), HPIM (b), and PIM (c).

5. Discussion

The activity-rest pattern is under the control of circadian clock and in our study, we observed the daily variations in the activity rest pattern in both healthy subjects and in the patients suffering with the upper-intestinal cancer (Figure 2). In general, higher activity during the daytime and lower activity during the night tine correlated with the diurnal nature of the human. Similar daily activity pattern is reported previously in healthy subjects; where healthy subjects (male and female) displayed a distinct and regular day-night pattern in wrist activity; where more activity was displayed during the day than night (Pati *et al*. 2007). In our study, we observed slow and steady increase in the general activity pattern with the sun rise and it achieves the maxima of activity during the middle of the day, however, higher activity was maintained both in the healthy subjects and in patients throughout the daytime and drop of activity was recorded after couple of hours later than the sunset. The minimum activity was recorded during the late phase of night. In previous studies conducted on cancer patients showed the effects of the disease conditions on the activity pattern. In contrast to our study, it has been reported that cancer patients show daytime napping and reduction in level and intensity of activity in comparison to healthy subjects (Pati *et al.* 2007).In our study, the cancer patients showed the daily variations in the activity with peak activity during the daytime. However, in the study conducted by the Pati *et al.* 2007 showed that impaired activity in cancer patients. Impairment of the daily activity-rest pattern has also been documented inpatients suffering from breast cancer (Roscoe *et al*. 2002), metastatic colorectal cancer (Chevalier *et al*. 2003; Mormont and Waterhouse 2002), lung cancer (Levin *et al*. 2005), and head and neck cancer (Pati *et al*. 2006).In our study, we observed that both the healthy subjects as well as cancer patients, exhibit a high amplitude 24 h rest-activity rhythm characterized by high level activity during the daytime (waking) hours and very low to zero-level activity during the night (rest). In general, there was no difference in the onset

and offset of activity timings of the healthy subjects and cancer patients. However, in the other studies this distinction was less marked in cancer patients (Chevalier *et al*. 2003; Levin *et al*.2005; Mormont and Waterhouse 2002; Pati *et al*. 2006; Pati *et al*. 2007).

Our study show that the rest-activity circadian rhythm is not easily altered, abolished or masked by cancer diseases, irrespective of stage, and performance status of the cancer. Similarly, earlier studies show the alteration in the magnitude of activity, but not the absence daily rhythmicity, in the characteristics of rest-activity or hormonal rhythms could vary as function of the severity of the disease (Bartsch *et al*. 1994; Mormont and Le´vi 1997; Mormont *et al.* 2000;Touitou *et al*. 1995). However, alterations in circadian rhythms have been reported in large tumor burden patients, liver metastasis, poor performance status, and advanced stage (Mormont and Le´vi 1997;Mormont *et al*. 2000).The activity performance status is frequently used to indicate the condition of patients and it can be an important factor in evaluating the effectiveness of treatment methods. Results from studies support this proposition and prove that the performancestatus of hospitalized cancer patients is positively correlated with their prognosis and duration of survival. Studies have identified a strong correlation between the performance status of cancer patients and rest activity rhythms and how it pertains to survival. Among patients with colorectal cancer, those with regular rest-activity rhythms and good performance status responded more favorably to treatment and had a better prognosis (Mormont *et al*. 2000). In our study we did not consider the performances of the cancer patients, however, daily profile was not much different in between the patients and healthy subjects.

In conclusion, the results of the current study found that cancer patients and controls exhibit 24 h rest-activity rhythmicity, with similar daily profile and amplitude.

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SECTION 3: TO STUDY THE SLEEP QUALITY IN CANCER PATIENTS

1. Introduction

Sleep quality is an important clinical construct for two major reasons. First, complaints about sleep quality are common; epidemiological surveys indicate that 15-35% of the adult population complain of frequent sleep quality disturbance, such as difficulty falling asleep or difficulty maintaining sleep (Karacan *et al*. 1976, 1983; Bixler *et al*. 1979; Lugaresi *et al*. 1983; Welstein *et al*. 1983; Mellinger *et al*. 1985). Second, poor sleep quality can be an important symptom of many sleep and medical disorders. One frequently measured component of sleep quality, sleep duration, may even have a direct association with mortality (Kripke *et al*. 1979). Sleep quality complaints are particularly relevant to psychiatry. Factors relating to anxiety and stress are one of the most important concomitants of sleep complaints in the general population (Karacan *et al*. 1983), and insomnia associated with psychiatric disorders is the most prevalent type of insomnia seen in sleep disorders centers, accounting for 35% of diagnoses (Coleman 1983).

Sleep disturbance is a common component of the cancer experience in adult and child cancer patients, survivors, and caregivers. There is beginning evidence that some aspects of sleep disturbance may contribute to the development of cancer and substantial evidence that sleep disturbance is associated with many aspects of cancer treatment, cancer symptoms and morbidity, mortality and quality of life. Although there is growth in understanding of the importance of sleep and its relevance cancer care, there is a continued need to improve scientific knowledge about the relationships among sleep disturbance, cancer biology, and the experience of cancer. Sleep disturbance and sleep-related symptoms are also well-

documented contributors to poor function (Willette *et al*. 2009) and quality of life in cancer patients (Liu *et al*. 2013).

Fatigue, a frequent outcome of sleep loss, is common in cancer patients and often studied by cancer researchers. A number of well-validated fatigue instruments are available, including several constructed specifically for cancer populations e.g., Brief Fatigue Inventory (Mendoza *et al*. 1999); Cancer Fatigue Scale (Okuyama *et al*. 2000); and the Functional Assessment of Chronic Illness Therapy-Fatigue (Cella *et al.* 2002). Because sleepiness and fatigue are overlapping, but distinct constructs, both fatigue and sleepiness should be measured as consequences of sleep loss, sleep disorders and/or systemic disturbance, rather than using one as a proxy for the other (Pigeon *et al*. 2003).

Sleep is a multi-dimensional phenomenon having distinct and measurable biological, behavioral, perceptual, and temporal (circadian, infradian, ultradian) properties. Sleep parameters are under regulation of behavioral and physiological processes that are the mechanisms through which sleep disturbance influences cancer, recovery, quality of life, and morbidity and mortality. Cancer therapies, cancer symptoms, and psychological and behavioral stressors associated with cancer and its treatment contribute to sleep disturbance that, in turn, may contribute to morbidity, mortality, and decrements in function and quality of life. While the importance of sleep is becoming more widely recognized, efficacious interventions are needed (Howell *et al*. 2014). Growing evidence suggests that excessively long or short sleep duration (Jiao *et al*. 2013) may increase the risk for developing cancer; and sleep-wake alterations may contribute to cancer mortality (Chang and Lin 2014). However, the mechanisms are not well-known.

There are growing efforts to understand the relationships between the epigenetics of sleep, circadian rhythms, and cancer biology (Qureshi and Mehler 2014). Sleep quality is

closely tied with the pain response (Affleck *et al*. 1996; Haack *et al*. 2007). While it is often assumed that poor sleep quality is a result of pain, the converse is often also true: Poor sleep, included sleep fragmentation and decreased rapid eye movement (REM) and slow wave sleep, leads to decreased pain thresholds and increased pain perception (Affleck *et al*. 1996; Onen *et al*. 2005). Sleep disturbance is a well-documented contributor to daytime fatigue in cancer (Jim *et al*. 2013; Minton and Stone 2012; Jacob *et al.* 2007; Roscoe *et al*. 2007;Stepanski *et al*. 2009) and daytime sleepiness among cancer patients. It is important to recognize that fatigue and sleepiness are distinct constructs; fatigue may contribute to sleepiness (Stepanski *et al*. 2009) and vice versa (Ahsberg and Furst 2001), but despite the overlap, distinguishing the two phenomena is necessary to guide clinical care (Pigeon *et al*. 2003). Sleepiness is usually related to sleep loss and specific sleep disorders associated with sleep loss, such as sleep apnea, while fatigue is more often associated with systemic conditions (Neu *et al.* 2010) and the presence of insomnia. Cancer patients seem more prone to disturbed sleeping patterns such as insomnia (defined as a subjective complaint of inadequate nocturnal sleep) (Passik *et al*. 2003; Graci 2005). Several other studies have reported that approximately half of patients with cancer suffer from sleep disturbances (Savard *et al*. 2001; Lindley *et al.* 1998).

Better understanding of the role of sleep in cancer development, progression, and quality of life, as well as clinical trials to test interventions to improve sleep and sleep-related outcomes is needed in the diverse populations of people who are cancer patients, survivors, and caregivers, as well as those who are at particular risk for cancer (e.g., shift workers).There are numerous approaches to measuring sleep, including polysomnography, actigraphy, and self-report questionnaires. To date, research on quality of sleep in cancer patients has been inadequate, as an effective measurement tool for clinical studies is lacking. There is a paucity of empirical data using psychometric measures about the prevalence and

nature of sleep disturbances in cancer patients, and there is no consistent use of measures with established reliability and validity. The Pittsburgh Sleep Quality Index (PSQI) is a self-report questionnaire, commonly used to assess quality of sleep. Although it has been considered appropriate in a variety of clinical populations, it has found limited use in cancer patients (Beck *et al*. 2004).

Daytime sleepiness is another common sleep related clinical problem, suggesting a serious underlying physiological anomaly (Ruggles *et al*. 2003). Daytime sleepiness is tend to be associated with higher mortality (Hays *et al.* 1996; Newman *et al.* 2000), increased risk for motor vehicle abnormalities (Maclean *et al.* 2003)and work-related accidents, and a higher prevalence of other diseases such as diabetes, myocardial infarction, and stroke (Chasens *et al.* 2009). Estimation of an individual's level of DS is important for better understanding the factors associated with the level of DS and to access the health and social consequences of DS. The Epworth sleepiness scale (ESS) measures the day time sleepiness and is most commonly used in sleep research and clinical diagnosis. The ESS was developed in 1991 using data from healthy subjects and patients with a variety of sleep disorders (Johns 1999). The ESS based on the peoples rating on a four-point scale, their usual chances of falling asleep in eight different situations, chosen to represent the different levels of somnificity that most people encounter as part of their daily lives (Johns 1999). Somnificity is the general characteristic of a posture, activity and situation that reflects its capacity to facilitate sleep-onset in the subjects (Johns 2002). The total ESS score is the sum of item-scores and it ranges between 0 and 24. Higher the value of score, higher the person's level of DS. From the sleep propensity viewpoint, each of the eight ESS item-scores represents a different subjectively-reported SSP (Johns 1994).

2. Materials and Methods

We used The Pittsburgh Sleep Quality Index (PSQI)and The Epworth Sleepiness Scale (ESS) to measure the sleep quality in cancer patients.

The Pittsburgh Sleep Quality Index (PSQI) (Buysse *et al*. 1989), was used for the subjective assessment of sleep quality. The Pittsburgh Sleep Quality Index (PSQI) was developed with several goals: (1) to provide a reliable, valid and standardized measure of sleep quality;(2) to discriminate between "good" and "poor" sleepers; (3) to provide an index that is easy for subjects to use and for clinicians and researchers to interpret; and (4) to provide a brief, clinically useful assessment of a variety of sleep disturbances that might affect sleep quality.

The PSQI asses sleep quality during the previous month. This is the time frame intermediate between post-sleep inventories (which asses only the previous night's sleep) and survey type questionnaires (which asses difficulties over the previous year or more). The PSQI consists of 19 self-rated questions and five questions rated by the bed partner or roommate. The PSQI yields seven sleep components related to sleep habits including duration of sleep, sleep disturbance, sleep latency, habitual sleep efficiency, use of sleep medicine, daytime dysfunction due to sleepiness, and overall sleep quality. These 19 items are grouped into seven component scores, each weighted equally on a 0-3 scale. The seven component scores are then summed to yield a global PSQI score, which has a range of 0-21; higher scores indicate worst sleep quality.

The PSQI is a widely used tool in research studies and clinical trials, and has been translated to several languages including German, Spanish, Chinese, and Hebrew, with comparable reliability and validity values (Shochat *et al*. 2007).

PSQI SEVEN COMPONENT SCORE

Sleep Quality Score: Sleep quality score represents the quality of sleep, higher the score poor is the quality of sleep.

Sleep Latency Score: Sleep latency score is the length of time that it takes to accomplish the transition from full wakefulness to sleep (i.e. how long it takes to fall asleep).

Sleep Duration Score: Typically refers to total amount of sleep obtained, either during the nocturnal sleep episode or across the 24h period.

Sleep Efficiency Score: It is the ratio of total time spent asleep (total sleep time) in a night compared to the total amount of time spent in bed (i.e. percentage of time in bed that one is asleep).

Sleep Disturbances Score: A disorder of sleep pattern which may be sever enough to interfere with a person's normal physical, mental and emotional functioning.

Sleep Medication Score: Taking medicine (prescribed or over the counter) to improved sleep quality.

Sleep Dysfunction Score: Any disorder that affects, disrupt or involves sleep.

THE EPWORTHSLEEPINESS SCALE (ESS)

The Epworth Sleepiness Scale (ESS) is used to determine a person's level of daytime sleepiness. Each item describes a routine daytime situation. Measures the propensity to doze or fall asleep during 8 common daily activities, such as: sitting and reading; watching television; sitting inactive in a public place; as a passenger in a car for an hour; sitting and talking to someone; sitting quietly after a lunch without alcohol; or in a car, while stopped for

a few minutes in traffic. Propensity for dozing is rated for each situation on a 4-point scale, from 0, indicating “would never doze,” to 3, indicating a “high chance of dozing.” Adding the scores for each of the 8 questions yields a total score ranging from 0-24. A score of 10 or greater indicates a possible sleep disorder.

3. Statistical analysis

Data represented as mean ± SE. Student t- test was used to determine the effect of health conditions (patients vs healthy subjects) on different sleep quality parameters. Significance was taken at $p < 0.05$.

4. Results

Various sleep parameters studied in the present study and their comparison with the healthy subjects is shown in the figure 3. In general, there was poor sleep quality (P = 0.0016; Student t-test; Figure 3a) in the patients in comparison to the healthy subjects (mean score of sleep quality in healthy subject: Mean ± SE: 0.85± 0.08; mean score of sleep quality in patients: Mean ± SE: 1.44± 0.14). Sleep efficiency was also compromised in the patients and was significantly reduced in comparison to the healthy subjects (P = 0.0205;Student t-test mean score of sleep efficiency in healthy subject:Mean ± SE: 0.01± 0.00; mean score of sleep efficiency in patients:Mean ± SE: .52± 0.19; Figure 3d). Sleep disturbances were more scored in patients in comparison to the healthy subjects (P = 0.0007;Student t-test mean score of sleep disturbances in healthy subject:Mean ± SE: 1.00± 0.00; mean score of sleep disturbances in patients:Mean ± SE: 1.36± 0.09; Figure 3e). Higher sleep medication score (P = 0.0017;Student t-test) was recorded in the patients (mean score of sleep medication in healthy subject:Mean ± SE: 0.05± 0.001; mean score of sleep medication in patients:Mean ± SE: 0.56± 0.10; Figure 3f) and sleep dysfunction was common in the cancer patients (P = 0.0043; Student t-test) in comparison to the healthy (subjects mean score of sleep dysfunction in healthy subject:Mean ± SE: 1.00± 0.01; mean score of sleep dysfunction in patients:Mean ± SE: 1.44± 0.01; Figure 3g). However, sleep latency score was higher (P = 0.0230; Student t-test) in the healthy subjects in comparison to the cancer patients (subjects mean score of sleep latency in healthy subject:Mean ± SE: 2.20± 0.18; mean score of sleep latency in patients:Mean ± SE: 1.64± 0.15; Figure 3b) and there was no effect of health condition on the sleep duration score (P = 0.5180;Student t-test; Figure 3c).

The Epworth Sleepiness Scale (ESS) suggest that there is a higher daytime sleepiness in the cancer patients in comparison to the healthy subjects (P = 0.0036; Student t-test; Figure

4). In cancer patients the mean ESS score was 7.2± 0.3 while in healthy subjects it was recorded 4.0± 0.3.

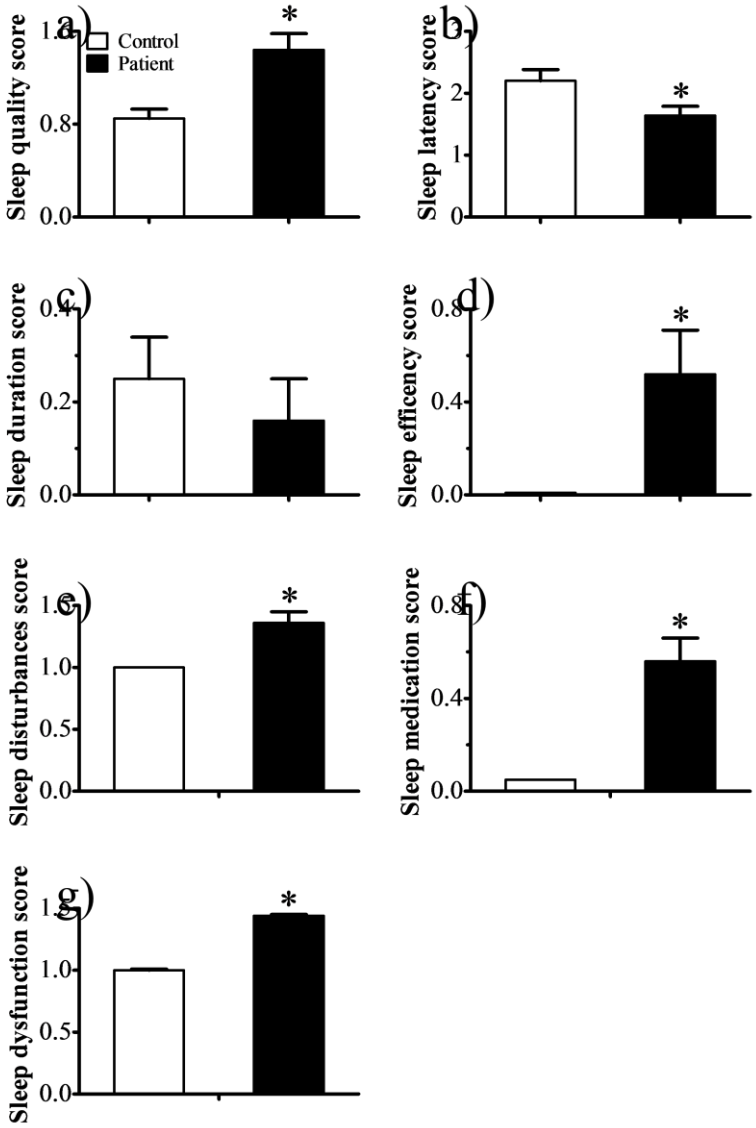

Figure 3. Various sleep parameters studied in the upper intestinal cancer patients represented by solid bar and their comparison with healthy subjects represented by hallow bar. Data is represented as mean ± SE. Sleep quality score (a), sleep latency score (b), sleep duration score (c), sleep efficiency score (d), sleep disturbances score (e), sleep medication score (f), and sleep dysfunction score (g). Asterisk (*) indicates a significant difference between healthy subjects and cancer patients ($p < 0.05$, Student t test).

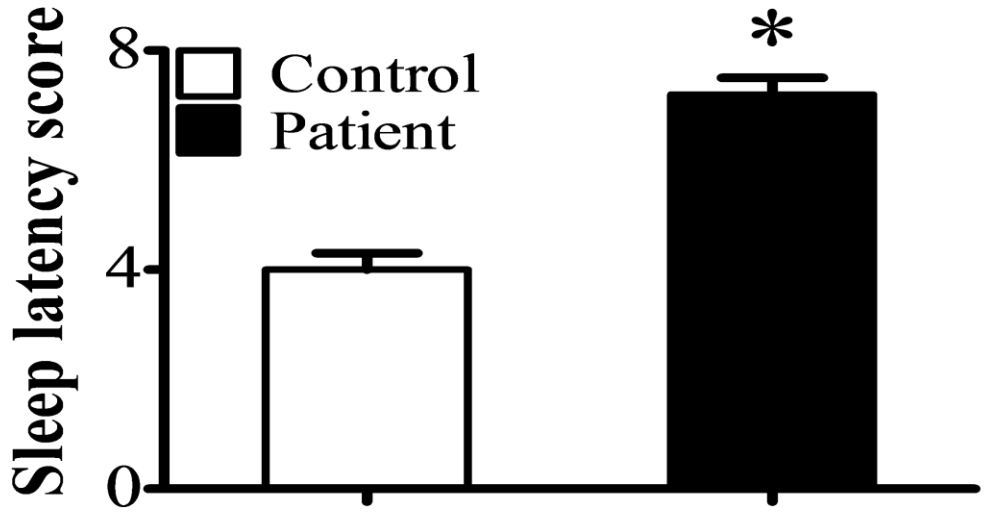

Figure 4. The Epworth Sleepiness Scale (ESS) between healthy subjects represented as hallow bar and cancer patients represented as solid bar. Data is represented as mean ± SE. Asterisk (*) indicates a significant difference between healthy subjects and cancer patients ($p < 0.05$, Student t test).

5. Discussion

Sleep/wake disturbances, particularly insomnia, are among the one of the common complaints of cancer patients, sleep problems have received little attention from healthcare providers (Savard and Morin, 2001) and information about individual sleep parameters in cancer patients is limited. In our study PSQI clearly reflects that the various sleep parameters are affected in the cancer patients in comparison to the healthy subjects. Our study is in consistent with the findings of Levin *et al.* (2005), where higher PSQI score were recorded in the patients with advanced non-small-cell lung cancer. In our study we also found that higher score of sleep quality, sleep efficiency, sleep disturbance, and sleep dysfunction clearly suggesting the poor performances of sleep parameters by the cancer patients in comparison to the healthy subjects. Higher nap frequency, total nap duration, average nap, and total nap duration per 1 h awake-time and longer sleep duration are reported in cancer patients than control subjects (Pati *et al*. 2007). However, in our study we did not find any effect of health condition on the duration of sleep (Figure 3c). We observed higher sleep latency score in the healthy subjects suggesting taking longer time to go to sleep in comparison to the cancer patients. Further, sleep medication score is also higher in the patients in comparison to the healthy subjects, suggesting use medicine for sleep and hence the time taken to go to actual sleep (sleep latency) is much lower in the cancer patients in comparison to the healthy subjects.

The various studies suggest that cancer patients have twice the prevalence of sleep problems as that reported in the general population (Savard *et al.* 2003), with most of people reporting maintenance insomnia with several awakenings during the night (Davidson *et al.* 2002; Lee 2003). Insomnia has been linked with higher rates of medical and psychiatric illnesses and poor quality of life (QOL) (Sateia and Pigeon 2004).Sleep/wake disturbances occur during all phases of cancer care (Clark *et al.* 2004; Gibson *et al.*2005; Hockenberry-

Eaton *et al*. 1998; Vena *et al.* 2004). Sleep/wake disturbances occur before the beginning of the treatment (Ancoli-Israel *et al*. 2001) and often seem to be directly linked to the cancer diagnosis (Lee *et al.* 2004; Savard *et al*. 2001). Problems with insomnia, and other nocturnal sleep disturbances, hence daytime fatigue are also common among caregivers of people with cancer and other chronic illnesses (Carter 2003; Carter and Chang, 2000; Hinds *et al*. 1999; Jepson *et al.*1999; Kozachik *et al.* 2001; McGrath *et al*. 2004;Nijboer *et al.* 2000; Nijboer *et al*. 1999). Patients with advanced non-small-cell lung cancer show distorted circadian rhythms and patients experienced higher than expected levels of wakefulness during the normal sleep period and extensive sleep periods during normal times for activity (Levin *et al*. 2005).

Age-dependent alterations in sleep have been reported in human subjects due to the weaker circadian regulation of sleep and wakefulness (Cajochen *et al*. 2006). However, lifestyle also influence the sleep performances (Monk *et al*. 2006). Sleep-wake disturbances in patients suffering with head and neck cancer (Pati *et al.* 2006) and colorectal (Chevalier *et al*. 2003) cancer have been documented. However, in comparison to our study, these studies do not report the effects on sleep efficiency, sleep latency, and actual wake time in cancer patients in comparison to the healthy subjects, but report the other sleep parameters (i.e., time in bed and assumed and actual sleep times) which differ in a statistically significant manner. In contrast to control subjects, cancer patients spent a longer duration in bed and exhibited longer assumed and actual sleep. Fernandes *et al*. (2006) also documented a significant difference in several sleep parameters, namely % sleep, sleep efficiency, and wake after sleep onset, between cancer patients and healthy volunteers. Other reports also documented that cancer patients tend to have fragmented sleep and poorer sleep efficiency, exhibit more restlessness at night, and take longer time to fall asleep (Ancoli-Israel *et al*. 2006; Berger 1998; Miaskowski and Lee 1999; Mormont *et al*. 1996). The cancer patients exhibited more

episodes of wake and sleep bouts during the habitual sleep period and longer time in bed and longer assumed sleep are a usual phenomenon. Both the frequency and duration of naps during the awake-span were higher among the cancer patients. Daytime naps were reported in patients with bone metastasis and breast cancer (Ancoli-Israel *et al*. 2006; Miaskowski and Lee 1999). Together, these alterations could lead to circadian rhythm alterations characterized by decreased amplitude and low 24 h average activity.

In our study, we observed higher ESS score for cancer patients in comparison to the healthy subjects, suggesting the daytime sleepiness is higher in cancer patients in comparison to the healthy subjects however, the total ESS score of cancer patients (7.2± 0.3). As in ESS score more than 10 is indicator of the sleep disorder therefore these cancer patients may not be experiencing major sleep disorders.

The much-needed research on sleep/wake disturbances in people with cancer needs to build on the extensive body of knowledge that exists regarding sleep/wake disturbances in the general population and specific sleep disorders such as sleep apnea, periodic leg movement syndrome, restless leg syndrome, narcolepsy, and insomnia.

6. References

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APPENDICES

Papers Presentation at Seminars /Workshop / Conferences:

- Presented poster entitled **"Suppression of cardiovascular rhythms in the patients suffering with upper-intestinal cancer"** (9th November, 2017) organized by Department of Biotechnology, School of Life Sciences, Mizoram University (A Central University), Aizawl-796004.

Seminars / Training / Workshop / Conferences Participated:

- Workshop on **"Mechanisms of adaptation in the Temporal Environment"** (May 23, 2017) organized by the Department Zoology, Mizoram University, Aizawl, Mizoram.
- Outreach Program on **"Human Health and Biological Timing''** (May 22, 2017) organized by the Department of Zoology, Mizoram University, Aizawl, Mizoram.
- Workshop on **"National Level Workshop on Biostatistics and Bioinformatics"** (1th– 7th September, 2016) organized by the Department of Biotechnology, Mizoram University.
- Workshop on **"Science Communication Workshop (SciComm 101)"** (6th June, 2017) organized by the Department of Biotechnology, Mizoram University.