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Insomnia and Sleep Deficiency in Pregnant Women: A Potential Study with Two Contrasts

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Abstract

Insomnia in pregnancy is associated with depression, preeclampsia, gestational diabetes, and preterm labor. In normal pregnancies, maternal plasma melatonin levels increase significantly as pregnancy proceeds, reaching its peak near term. However, the role of melatonin in insomnia during pregnancy is not known. The objective of this study was to measure nocturnal saliva melatonin levels in pregnant women with and without insomnia. Results did not show a significant difference in melatonin levels between insomniac (treated and untreated) and healthy pregnant women in all trimesters. However, sub-group analysis showed significantly lower melatonin levels in untreated insomniac pregnancies compared to healthy pregnancies and those treated with sleep medications. Results of this study confirmed lower levels of nocturnal melatonin in untreated pregnancies with insomnia. Future research is needed to investigate the safety and efficacy of melatonin supplementation for the treatment of insomnia in pregnancy, replacing psychotropic drugs.

Keywords: Insomnia depression, preeclampsia, gestational diabetes, preterm labor

Introduction

Circadian System

The circadian system is located both in the central nervous system in the suprachiasmatic nucleus (SCN) of the hypothalamus, and peripheral organs such as the heart and liver.[1] The system plays an important role in different physiological arrangements and behavior. It is the biological clock of the body managing both night and day behaviors. The system functions not only independent of external stimuli such as ambient brightness, but also in a manner dependent on external factors such as food intake.[2] Any disruption of the system leads to conditions such as sleep disorder, glucose intolerance (diabetes), obesity, and ultimately decreased life expectancy.[3]

Suprachiasmatic Nucleus

SCN is located in the anterior hypothalamus on top of the optic chiasm, next to the third ventricle with 50,000 neurons.[4] It is a bilateral structure and is activated by light. In other words, it regulates the circadian rhythm. Although it is now known that circadian rhythm exists in peripheral organs independent of the SCN, their function is lost without input from SCN. For this reason, SCN is called the master circadian clock, or the coordinator of the clocks. A circadian clock has a 24-hour cycle and its most potent stimulus is light that is directly projected from the retina to SCN through the retino-hypothalamic tract (RHT). The indirect pathway of receiving light by the SCN is through the intergeniculate tract (GHT).

Malfunctioning of any of the above pathways impairs the phase-shifting and light-dark cycle of the circadian system. For example, the light-dark cycle is impaired by the RHT pathway damage, and phase-shifting is delayed by damage to GHT pathway.[1] Research also shows that the circadian clock can shift a phase independent of light with other stimuli such as exogenous melatonin, temperature, exercise, and food. For example, melatonin supplementation in blind people can regulate the circadian clock.[5] Core body temperature also affects the peripheral circadian clock but not the SCN.[6] There are 2 pathways by which the SCN manages the circadian clock complementing each other: endocrine and neural pathways. Circadian clock controls the endocrine system including melatonin, cortisol from the hypothalamic-pituitary- adrenal (HPA) axis, the hypothalamic-pituitary-thyroid (HPT) axis, and epinephrine.[7] The neural pathway includes projections from the SCN to the pineal gland (Figure 1).

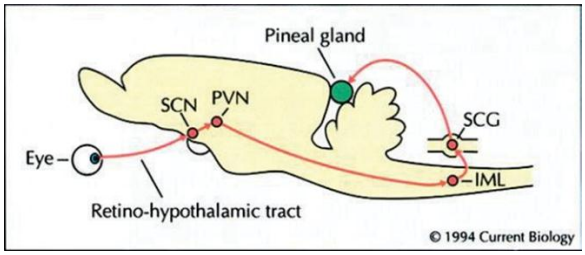

Figure 1: The neural pathway between the SCN and pineal gland in rats. Reprinted with permission from Springer.[46]

Pineal Gland

Description

The pineal gland had several names over-time given its anatomy and function in different animals. It was first identified as a different cerebral organ a few centuries BC, and was initially named "Konareion" for its pine shape, similar to lymph nodes. It was then named "Glandula Pinealis", pineal. Later Rene Descartes called the gland the "seat of the soul", coordinator of psychophysiological functions of the body. Bioassay advances resulted in the discovery of pineal "extracts" responsible for lightening frog's skin, which lead to the isolation of melatonin in 1958. Development of fluorescent techniques allowed the measurement of melatonin and serotonin, their light-dark cycles and their concentrations.[8]

Structure

The location and structure of the pineal gland is species specific and responds to environmental stimuli. The human pineal gland is located at the posterior of the diencephalon. It develops in the second month of gestation as an invagination of the ependymal lining of the diencephalic third ventricle, between the habenular and posterior commissure. The "pineal stalk" consists of a rostral and a caudal lamina surrounded by a pial layer, and is suspended in the CSF- filled pineal recess below the splenium. Average adult dimensions are 5-9mm in length, 1-5mm in width, and 3-5mm in thickness and the average weight of 100-180mg varies in different ages and genders.[8] Its structure is described as two parts. A central core of lobules and peripheral neurons along with pinalocytes are the main cell types of human pineal gland. Pinalocytes are granular shape, and have a nucleus and cytoplasmic processes that end in capillaries, indicative of an endocrine gland. Neuroglia are distributed unevenly in the periphery. Calcareous deposits are characteristic of the pineal deposits as calcium and magnesium salts on both parenchymal and intracellular tissue. Calcareous deposits are present from birth and increase in concentration by age, starting to decrease in young adulthood. A negative correlation was found between the density of calcification, age and chronic sleepiness. The pineal gland has a rich vascular system receiving blood from posterior choroidal arteries deriving from cerebral arteries. It lacks an endothelial blood-brain barrier, and is sensitive to drugs. The gland is innervated with fibres from sympathetic, parasympathetic, and central nervous systems. The most important pathway is the noradrenergic sympathetic pathway neurons which receive input from the suprachiasmatic nucleus (SCN) of the hypothalamus, which itself receives input from retinal ganglion cells.[8]

Neural Pathway

The neural pathway regulating the production of melatonin in the pineal gland consists of an input from the GABAergic system from SCN to a subdivision of paraventricular nucleus (PVN), which in turn projects to the intermediolateral (IML) cell column and, by pre- ganglionic adrenergic fibres, to the superior cervical ganglion (SCG). The input then reaches

the pineal gland from SCG by post ganglionic adrenergic fibres and releases norepinephrine (NE).[35,36] In the pineal gland, beta adrenergic receptors of pinalocytes are stimulated by a subunit of G protein coupled receptors that increase adenylate cyclase. Ca^{++} and protein kinase C (PKC) are involved in increased beta adrenergic activity and stimulation of cAMP potentiated by Alpha adrenergic receptors.[37] The synergistic activity of alpha and beta adrenergic receptors increases cAMP and production of NAT[38] (Figure 2). The rate of melatonin secretion is negatively correlated with age [39], and dim light melatonin onset (DLMO) may not reach the threshold concentration, 4 pg/ml, in the elderly.[40] However, concentration is significantly higher in younger people.[41] There are also inter-individual differences in timing, duration, and the amount of nocturnal hormone secretion[42], but stability within individuals.[43,44] For example, mean melatonin production is estimated to be higher in men than women at night, 60.7± 24.6 pg/ml and 29.3± 21.8 pg/ml respectively, $p<0.01$.[45] DLMO also occurs later in women than in men.[41] Inter-individual differences in melatonin production may be due to different metabolism rates.[46]

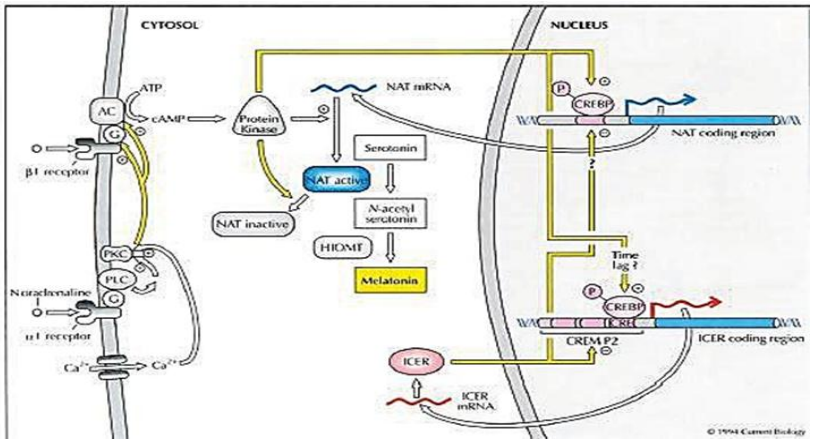

Figure 2: Adrenergic pathway of melatonin biosynthesis. Reprinted with permission from Elsevier. [47]

Day length affects melatonin secretion as well, so there are seasonal variations in the rate of nocturnal melatonin, which affects the physiology, behavior, and reproduction of some species.[9] The use of artificial light or artificial shortening of summer days has been shown to affect the secretion of the hormone.[48,49] Evidence shows that the onset of "seasonal affective disorder" is related to shorter days and longer nights in fall and winter.[50]

Evidence of melatonin as the circadian phase maker comes from studies showing shifts in body temperature and sleep timing following melatonin administration. Therefore, supplementation with melatonin at the right time can adjust circadian rhythm after a phase-shift due to shift work, jetlag, blindness, or sleep disorders.[51] Evidence shows strong association between melatonin, sleep, and body temperature, energy, activity, and mental ability. The daytime low level of melatonin and high level of cortisol mediate daytime activities. Sleep and the dark-light cycle are also mediated by changes in melatonin and cortisol levels.[52] The rise of melatonin two hours before bed allows for sleep onset, which in turn decreases the nocturnal body temperature.[17] Studies show that blocking the beta adrenergic receptors suppresses nocturnal melatonin, and increases body temperature. This can be reversed by administering 5 mg melatonin. This further confirms the role of melatonin in body temperature regulation.[53] Melatonin levels suppressed either pathologically or artificially by light lead to insomnia (initiation, maintenance, sleep quality).[54] Figure 3, below shows the immediate effect of light exposure on nocturnal melatonin levels.[55]

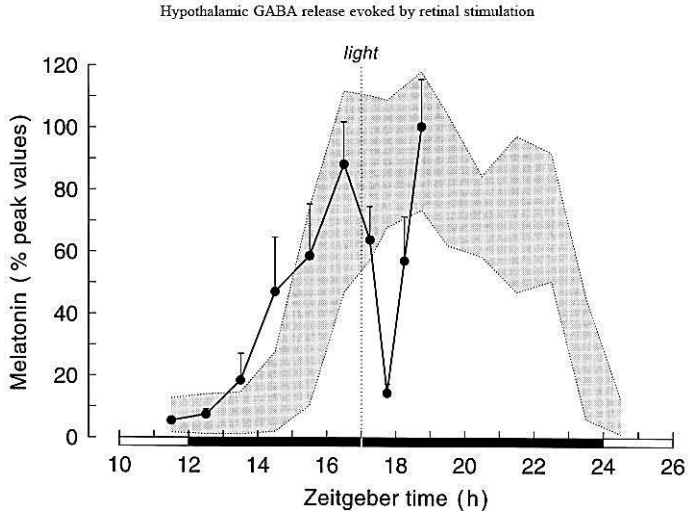

Figure 3: Effect of light exposure on nocturnal melatonin concentration. Normal pattern of melatonin synthesis in dark environment is shown in grey area. Reprinted with permission from Elsevier.[55]

Sleep

Sleep is defined as a state characterized by a reduction in voluntary movement, reduced response to stimuli and, ultimately, loss of awareness.[52] Sleep is divided into two parts: non-rapid eye movement (NREM) and rapid eye movement (REM). NREM itself is divided into three stages, which progress to the deeper sleep phase, or REM.[56] Sleep restriction which reduces sleep duration affects the structure of sleep.[57] Regulation of sleep in the brain is a complicated process involving several growth factors synthesized in response to neural activity. These growth factors also affect synaptic efficacy. Interlukin-1 (IL-1) and tumor necrosis factor (TNF) are the two most important factors in the sleep regulation process, released by the production of ATP after neurotransmission. They increase NREM sleep. Inhibition of these two compromises spontaneous sleep and rebound sleep after an episode of sleep deprivation. After sleep deprivation, IL-1 and TNF concentrations rise. However, their over-activation, due to the activation of innate immune response, inhibits sleep.[58] Other factors leading to this up regulation are light and excessive food intake. IL-1 leads to production of corticotropin releasing hormone (CRH) which, in turn, inhibits sleep dependent IL-1 inhibition. CRH also inhibits IL-1 which causes sleep inhibition. Other growth factors involved in sleep regulation are nitrous oxide (NO), growth hormone releasing hormone (GHRH) and nerve growth factor. Nuclear factor kappa B (NFkB), adenosine, and prostaglandin. NFkB is activated by sleep deprivation. Activation of NFkB leads to the activation of adenosine receptor, COX2, and NO synthase. COX2 leads to the synthesis of sleep promoting prostaglandin D2 (PGD2) and sleep inhibiting E2 (PGE2) that inhibits IL-1. Therefore, several positive and negative feedback loops are involved in sleep regulation or dysregulation. The neural system defined above affects the synaptic efficacy and downstream pathways that lead to wakefulness and consolidation of memory, or sleepiness.[59]

Insomnia

Insomnia, a form of sleep restriction, is an important public health issue.[60] A large population- based study calculated the prevalence of insomnia to be 6 - 48% after all diagnostic criteria were brought into consideration.[61] It is defined as an inability to initiate sleep, waking up during the night, difficulty falling back to sleep, and non-restorative sleep.[62] Delayed sleep phase disorder (DSPD) is a disorder of circadian rhythm and insomnia, in which the patient has difficulty initiating sleep at a normal time, therefore the circadian rhythm is delayed when compared to healthy individuals.[41] DSPD patients are able to maintain sleep if they go to bed in their favourite time, but sleep initiation is delayed. This type of insomnia is associated with daytime sleepiness and many physiological dysfunctions. Ultimately long term DSPD insomnia is associated with psychological disorders, substance and alcohol abuse, and other neurobehavioral and physiological disorders. The prevalence of DSPD is 7-16% in the general population.[63] To further understand the etiology of DSPD, nocturnal melatonin levels were measured in patients with this disorder and were compared with normal sleepers. Results showed that DSPD patients have significantly delayed dim light melatonin onset (DLMO) by three hours when compared with healthy sleepers (Figure 4). There was no difference in the duration of melatonin secretion but levels did not change significantly from DLMO to acrophase in DSPD patients.[64] DLMO is considered an approximate night-time threshold of melatonin synthesis and secretion. DLMO makes an assessment of the circadian rhythm disorder in patients with insomnia easier than a provisional assessment. 10 pg/ml is considered the threshold for plasma DLMO and 3 pg/ml for saliva (Figure 6).[65] Melatonin secretion starts at darkness and is suppressed by light, so it is very important to measure hormone levels in dim light to determine its onset time (DLMO) and to assess circadian rhythm in advanced sleep phase disorder (ASPD) or delayed in DSPD[41]. DLMO can be measured in both blood and saliva. Although DLMO measurement is currently used in research, there is no approved assay or standards in clinical settings.[66]

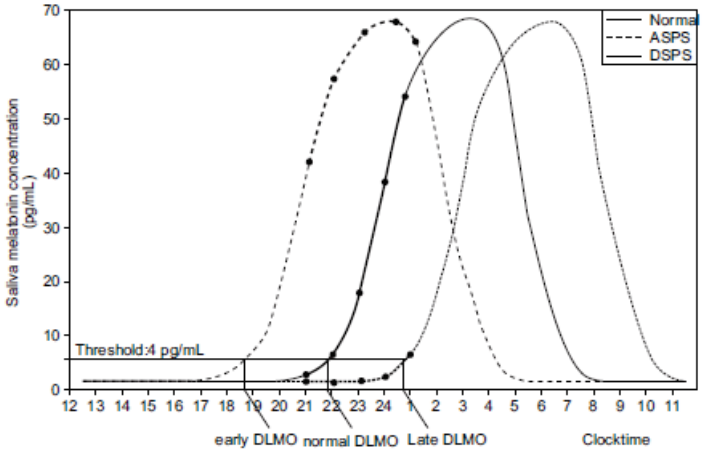

Figure 4: Pattern of melatonin secretion in a normal (solid line), ASPS (dashed line), and DSPS (dotted line) subject. Reprinted with permission from Elsevier.[41]

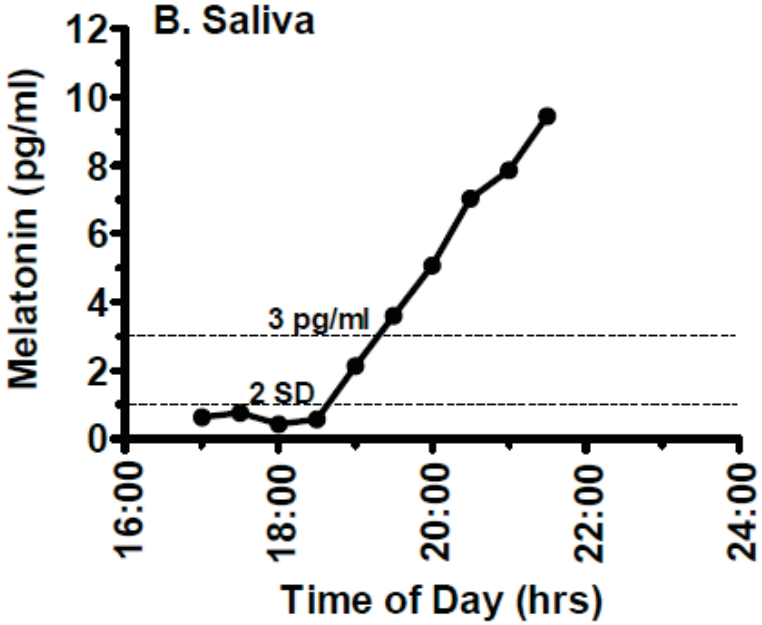

Figure 5: DLMO levels at 3 pg/ml in saliva. Reprinted with permission from the Journal of Clinical Sleep Medicine.[65]

Physiological Impacts of Insomnia

Chronic medical conditions were reported in 76 - 83% of the population of different ethnic groups due to poor sleep pattern.[67] Adverse psychological and physiological effects of insomnia have a major impact on the quality of life of affected individuals. Recent experimental and epidemiological data have associated insomnia with multiple daytime symptoms and chronic conditions. Among physiological impacts of sleep disorders are metabolic disorders leading to obesity and type 2 diabetes.[68] Insufficient sleep contributes to weight gain via excessive food intake associated with increased energy expenditure. Studies show that sleep restriction reduces daytime leptin while increasing daytime ghrelin. These hormonal changes lead to increased hunger and appetite.[69] Increased food intake is beyond the energy needed leading to weight gain.[70] Glucose intolerance increases and its clearance decreases upon sleep loss due to an impaired metabolism system.[71] Studies suggest the potential benefit of recovery sleep.[70]

Hypertension is among other physiological side effects of sleep restriction. It is shown in several studies that insomnia and short sleep duration is associated with hypertension. Duration of sleep is a predictor of the severity of hypertension.[72] When adrenocorticotropic hormone (ACTH) and cortisol levels were compared in insomniac patients and normal sleepers in a lab environment, results were elevated nocturnal levels of those two hormones leading to hyper-activation of hypothalamic-pituitary-adrenal axis. This, in turn, causes several pathological consequences, such as hypertension.[73] Obesity, increased insulin resistance and cardiovascular diseases are associated with elevated levels of ACTH and cortisol. Irwin et al. showed an increase in nocturnal norepinephrine in insomniac patients when compared to depressed and healthy individuals. This may be an indication of increased sympathetic activity, exhibited by increased heart rate, body temperature and metabolic rate.[74] Other studies also showed coronary artery disease mortality after chronic insomnia in middle-aged men and women.[75]

Impacts of Insomnia in Pregnancy

There are physiological and psychological consequences following insomnia in pregnancy. Glucose intolerance and gestational diabetes mellitus (GDM) are amongst the physiological impacts of insomnia in pregnancy. The prevalence of GDM in the US is approximately 7%, affecting 200,000 women every year. Risk increases by obesity and/or type 2 diabetes (T2D).[40] Earlier it was mentioned that insomnia is a risk factor for type 2 diabetes. As a result, insomnia and T2D may increase the risk of a pregnancy to develop GDM. In a cohort study following up pregnant women from early pregnancy with their sleep duration, it was found that women with shorter sleep duration are at 1.86 fold increased risk for development of gestational diabetes.[41] GDM adversely affects both maternal and fetal health and pregnancy outcomes. Mothers with GDM are at increased risk for cardiovascular disease and metabolic dysfunction.[42] GDM put the pregnancy at risk for pre-eclampsia, preterm labor and caesarean section due to the accumulation of excessive amniotic fluid, and infection.[43] In addition to maternal risks associated with GDM, children of diabetic mothers are at increased risk for macrosomia, related birth injuries, respiratory problems, neonatal hypoglycemia, and jaundice.[44]

Hypertension is one of the other physiological impacts of insomnia in individuals. In a pregnancy prospective cohort study, mean diastolic, systolic and mean arterial pressures were measured. It was found that systolic blood pressure is highest in those who sleep less than six hours at night in both first trimester and third trimester, a 1.62 and 4.41 fold increase respectively. Also, diastolic blood pressure blood pressure was highest in short time sleepers in both first and third trimesters, 1.85 and 3.52 fold increase respectively. Mean arterial pressure was significantly higher in both short and long sleep durations. [45] In the previous chapter mechanisms responsible for hypertension associated with insomnia have been explained.

Changes in body's hemodynamics such as temperature, heart rate and hyper-activation of the HPA axis are all factors in increased risk for hypertension.

Circadian system dysregulation and insomnia is now known to be a risk factor in depression and mood disorders in pregnancy. In line with this, studies investigated plasma melatonin levels in pregnant women with and without depression. Levels were measured at baseline, synthesis onset, and offset times. It was found that daytime melatonin levels are significantly higher in depressed pregnant women, while night time levels were significantly lower when compared to healthy pregnancies. Melatonin synthesis onset time and offset time were also significantly earlier in depressed pregnant women. Second finding of this study was that the number of previous episodes of depression was negatively correlated with melatonin offset time r=-0.66.

Therefore, previous episodes of depression may be a risk factors in earlier onset and offset times of the hormone production and its low levels in pregnancy. Depression disorders in pregnancy are explained by loss of sensitivity of melatonin receptor to estradiol and progesterone levels (Figure 6). Therefore, rise in gonadal hormone in healthy pregnancy results in an increase in melatonin levels but not in depressed pregnancies.[46]

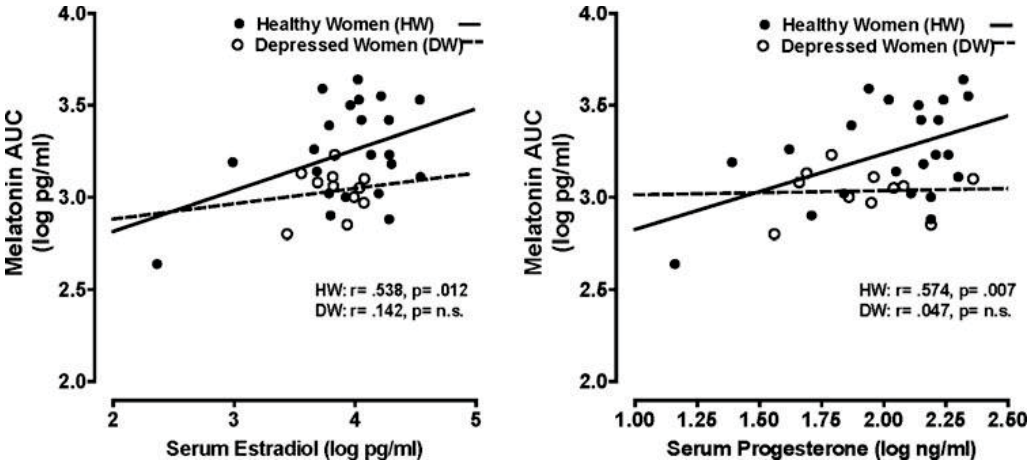

Figure 6: Relationship between melatonin levels and estradiol and progesterone in healthy and depressed pregnant women.Reprinted with permission from Journal of women's health, Mary Ann Liebert Inc[147]

Rationale

Sleep is important for the physiological and psychological wellness, quality of life, and overall well-being. Primary insomnia or insomnia secondary to depression and anxiety are major health issues amongst pregnant women.[74] Elevated blood pressure,[45] gestational diabetes,[41] preterm birth,[48] preeclampsia, prolonged labor, increased cesarean section rates, prenatal and post-partum depression are amongst adverse pregnancy outcomes associated with sleep disorders in pregnancy.[75] Poor sleep quality and quantity in the last month of pregnancy are associated with post-partum depression.[91]

Although there is a lot of epidemiological data on the impact of insomnia on pregnancy and its outcome, little is known about the pathophysiology involved. Understanding the pathophysiology and etiology of sleep helps address the issue with better treatment regimen.

Physiological and psychological impacts of untreated insomnia on maternal and fetal health and on pregnancy outcome lead to more in-depth investigation of the disorder and treatment options. Since melatonin is a naturally occurring hormone in our body and is responsible for the sleep-wake cycle and circadian rhythm, it is very important to find out if pregnant women with insomnia have lower levels of the hormone. To our knowledge no study has examined the effects of melatonin levels on the pathophysiology of insomnia.

In normal pregnancies, an increase in nocturnal maternal melatonin levels after 24 weeks to term has been documented.[28] Maternal and umbilical cord melatonin levels are highly and positively correlated when measured at term, suggesting that the hormone freely crosses the human placenta.[30] Local synthesis of the hormone by the human trophoblast have been identified as indication of autocrine, paracrine, intracrine and endocrine function of the hormone.[28,35] However, there is still a question of whether placental melatonin levels play a role in the sleep patterns of pregnant women.

The rationale for doing this study was to find out if salivary melatonin levels in pregnant women with insomnia were lower than in healthy pregnancies. An understanding of the melatonin levels in insomniac pregnancies aids in choosing a better treatment such as melatonin as a natural hormone therapy as opposed to psychotropic medications and their potential risk for the health of pregnancy and the unborn child. If lower melatonin levels are found it leads us to further research the safety and efficacy of melatonin supplementation to treat insomnia in pregnancy (without the side effects of pharmacotherapy on both mother and fetus/baby, which are explained in detail earlier in this paper). Melatonin is a natural hormone, and it was explained above that it has a high efficacy profile in treating insomnia with minimal side effects in non-pregnant individuals. To our knowledge there is no safety and efficacy study on the hormone in pregnancy. The rationale behind choosing saliva measurement was that it is non-invasive, and patients can collect the samples in the convenience of their home before bed. Melatonin was measured three times in pregnancy − at the end of all trimesters to get a better understanding of the pattern of increase, in each patient and between patients.

Objective of study

The objective of this study was to measure and determine salivary melatonin levels in pregnant

women with insomnia, treated or untreated, and to compare their levels with healthy pregnant women in all trimesters of pregnancy.

Methods

Measuring Melatonin in Saliva

Saliva

Healthy individuals produce 500-1500 ml of saliva per day, or 0.5 ml/minute salivary output, and composition may be different in terms of viscosity, ions and protein concentrations. The flow of the saliva depends on autonomic stimulation. Parasympathetic stimulation results in a large flow, with low levels of inorganic and non-protein organic compounds, such as cholesterol, uric acid, glucose, bilirubin, and creatinine. Sympathetic stimulation results in a small flow high in inorganic compounds such as K^+ and proteins. Compounds are released into saliva by different means, such as passive diffusion for lipophilic compounds, active transport and ultrafilteration through gap junctions.[76]

Saliva Melatonin and its Interactions

Melatonin transfers into saliva by passive diffusion.[76] Composition of the saliva is affected by food intake, increasing the release of total proteins. Caffeine should be avoided 12-24 hours before saliva sampling because it stimulates the activity of CYP 1A2.[77] Banana with low melatonin content is shown to increase urinary melatonin metabolite after consumption. This can be explained by the rate of absorption or metabolism of melatonin due to CYP1A2 polymorphism. Chocolate increases melatonin concentration by its flavonoid content.[178] Some drugs such as NSAIDS (ibuprofen, naproxen, diclofenac, ketorolac, etc.) may also reduce melatonin concentrations by inhibiting prostaglandin and COX-2.[79] Therefore, in the current study, patients were instructed to avoid the above mentioned to prevent any interaction with saliva melatonin levels.

Measuring Melatonin in Saliva

Salivary measurement of melatonin is now a practical and reliable tool for diagnosis of the condition and research purposes. Saliva melatonin as a circadian phase maker is validated and compared to plasma levels. There is a significant positive correlation between saliva and plasma melatonin onset r=0.64, and saliva and plasma acrophase concentrations, r=0.83 with saliva levels being one-third of plasma's (Figure 7). [80] Saliva sampling should be done in dim light and taken every 30-60 minutes for at least one hour to determine its onset.[81] Reliability of saliva melatonin measurement to determine dim light melatonin onset (DLMO) was assessed. Results showed that saliva measurement of melatonin levels is valid, and it is a reliable tool for the measurement of hormones.[81] This was further investigated by comparing in-home methods to laboratory measurements. Patients with insomnia were instructed to collect saliva samples at home and the following evening saliva samples were collected in the lab under lab conditions. It was found that at-home saliva melatonin levels are positively correlated with in-lab measurements r=0.85. However, a delay of approximately forty minutes was seen with the in- home method. This study confirmed the previous work that in-home saliva melatonin measurement is practical and valid to determine DLMO and to assess circadian rhythm (Figure 7).[66]

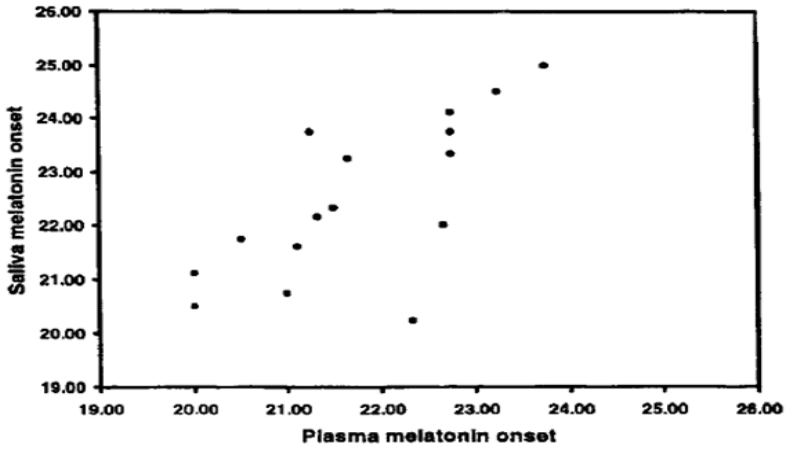

Figure 7: Relationship between plasma and saliva melatonin levels r=0.64. Reprinted with permission from SAGE publications.[180]

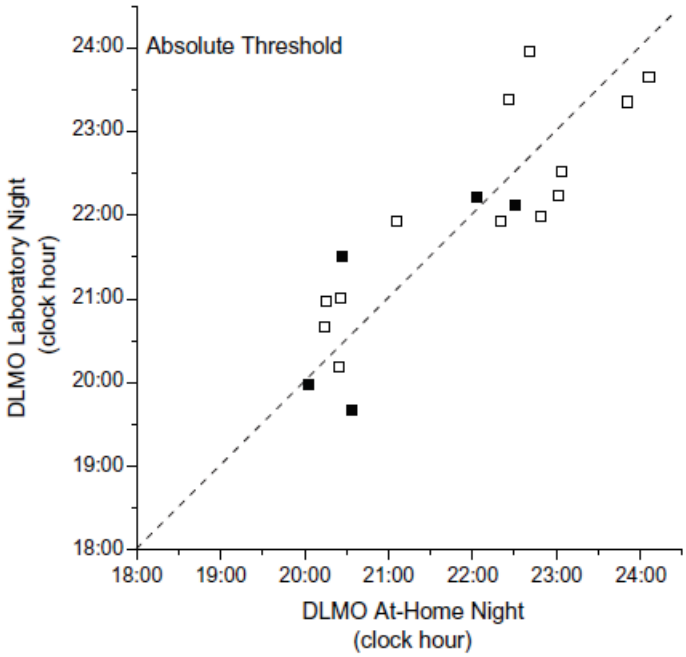

Figure 8: Relationship between in-home saliva melatonin and in-lab measurement, r=0.8. Reprinted with permission from Elsvier Limited.[66]

Study Methodology

Saliva collection was done three times during pregnancy; at 12 -14 weeks, 24-26 weeks, and 34-36 weeks. I was interested to know the pattern of melatonin rise from the beginning of pregnancy in all three groups described above. Plasma melatonin levels were measured in all trimesters[29]. Participants collected saliva samples 3 times every 30-60 minutes, in dim light, starting at least 1 hour prior to bedtime. Therefore, in total there were 9 saliva samples from each participant. No chocolate or bananas, alcohol, caffeine, or drinks with artificial colorants should have been taken on the day of sampling. No aspirin or medicines containing ibuprofen, would be taken on the day of sampling. The participant needed to remain in dim light during the sampling hours with a night light or a low wattage lamp. They should not have sat closer than 6 feet to a TV; and if using a computer/laptop/tablet, they should have adjusted the contrast to low. To avoid contamination with food, participants needed to finish their main meal at least 30 minutes before sampling time and to brush their teeth without toothpaste then rinse with water 10 minutes before sampling.[80] For saliva sampling a cotton swab was used, which was chewed or held in the mouth for 1-2 minutes, and then placed in a vial when soaked enough. Participants documented each sample time on a log provided to them along with the duration of staying in dim light. Participants were instructed to refrigerate or freeze their saliva samples within 30 minutes of sampling and mail them back in a cold box within 1-3 days. All storage and shipment accessories were provided to participants. The saliva samples were tested for melatonin levels using the enzyme-linked immunosorbent assay (ELISA) method in the Motherisk lab.

Statistical Analysis

All statistical analysis was done with student SPSS version 23. All data is presented in mean ± SEM. In order to compare melatonin concentrations between pregnant women with insomnia either treated or untreated (combined in one group) with healthy pregnant women a t-test was used. The null-hypothesis was that there was no difference in melatonin levels in insomniac pregnant women and healthy pregnancies.

For sub-group analysis, one-way ANOVA was used. Subgroups included pregnant women with treated insomnia, pregnant women with untreated insomnia and healthy pregnant women.In the general linear model, repeated measure analysis was used to compare the pattern of increase of melatonin levels in all two groups of untreated insomniacs, treated insomniacs and healthy subjects. Alpha was set at $p<0.05$ in all statistical analysis.

Results

In the first group, Exposed, a total of 24 people were recruited. 13 were lost to follow up. They did not respond to emails or phone calls after the ICF

was signed. Two had miscarriages and automatically were excluded from the study. One patient provided a very small amount of saliva sample that was not measurable. One patient's saliva melatonin measure was an outlier, and was deleted from the analysis. In the first trimester seven samples were used in (T1) melatonin measurement. In the second trimester (T2). This is while one patient withdrew after T1, but one patient was recruited in the second trimester due to her severe insomnia symptoms. In the third trimester (T3) measurement, there were five samples because two patients withdrew after T2.

In the second group, Disease-match, 19 people were recruited. Six were lost to follow up, and 13 samples were received for the T1 measurement. For the T2 measurement there were 12 samples, as one patient withdrew after T1. For the T3 measurement there were 9 samples because one patient withdrew. Two other patients had sampling dates much later than the expected end date of study.

In the third group, Healthy, a total of 28 people were recruited. 7 were lost to follow up, two had miscarriages and one provided small amount of saliva that was not measurable. A total of 18 samples were used for the T1 measurement. For the T2 there were 11 samples. There were 4 withdrawals, 2 miscarriages, and one was lost to follow up in T2. In T3, there were 12 samples, because one patient that was lost to follow up in T2 came back and continued the study (Figure 9).

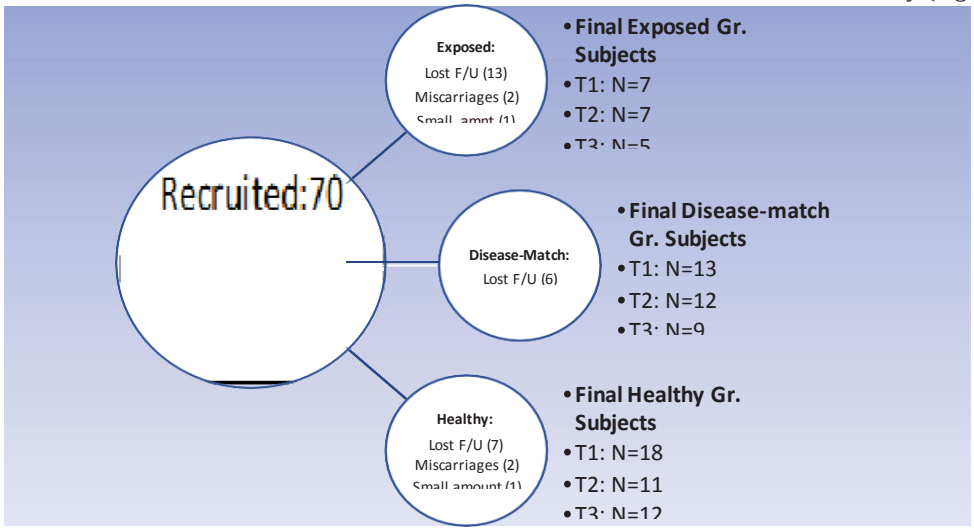

Figure 9: Study population saliva sample size per group per trimester.

Mean (SD) age was not significantly different among the groups, 35.43±3.7, 35.54±4.4, 33.73±3.7, respectively. Ethnic background, marital status, education, occupational status, number of pregnancies (gravidity), number of live children (parity), spontaneous abortions (SA), therapeutic abortion (TA), ectopic pregnancies (Ect), pre-pregnancy BMI, third trimester BMI, and total weight gain.

Disease Characteristics of the Insomnia Group

Five patients in the Exposed group suffered from insomnia only. One patient had insomnia, depression, anxiety and attention deficit disorder (ADD). One patient had insomnia, is bipolar, and had mania. One patient had insomnia and anxiety, and one patient suffered from insomnia and depression.

In the Disease-match group, eight patients suffered from insomnia only. One patient had insomnia, depression, and anxiety. One patient had insomnia and anxiety, two patients had insomnia and depression.

All nine patients in the Exposed (treated) group took medications for either sleep issues or comorbid symptoms such as depression and anxiety. Five patients took one or combinations of zopiclone, quetiapine, lorazepam, dimenhydrinate, and progesterone as a sleep aid. One patient took a combination of bupropion, amitriptyline, lisdexamphetamine, and clonazepam for insomnia, depression/anxiety, and ADD. Trazodone and aripiprazole were taken by another patient for bipolar disorder, mania and insomnia. Zopiclone, duloxetine and trazodone were used in combination for insomnia and comorbid anxiety. A combination of bupropion and quetiapine was taken for insomnia and comorbid depression (Table 1).

Condition	Exposed Group N=9	Disease-Match Group N=12
Insomnia Only	5(55%)	8(67%)
Insomnia with other psychiatric comorbidities	4(44%)	4(33%)

Table 1: Disease composition of exposed and disease-match group.

T-tests compared insomniac (exposed and disease-match) pregnant women (N=20) with healthy pregnant women. It was found that mean melatonin levels in all three trimesters were not significantly different between the two groups, p=0.4, p=0.9, p=0.1 for T1, T2, and T3 respectively (Table2) (Figure 10).

	Groups	N	Mean melatonin pg/ml	Std. Deviation	Std. Error Mean	P-value
T1	Insom. H	20 18	9.4 11.0	6.1 6.3	1.3 1.5	0.4
T2	Insom. H	19 11	11.9 11.9	12.6 11.7	2.9 3.5	0.9
T3	Insom. H	14 12	11.3 17.4	6.5 12.0	1.7 3.4	0.1

Table 2: Descriptive statistics and t-test results. No significant difference found between the groups in all trimesters. T1: first trimester, T2: second trimester, T3: third trimester, Insom: insomnia, H: healthy

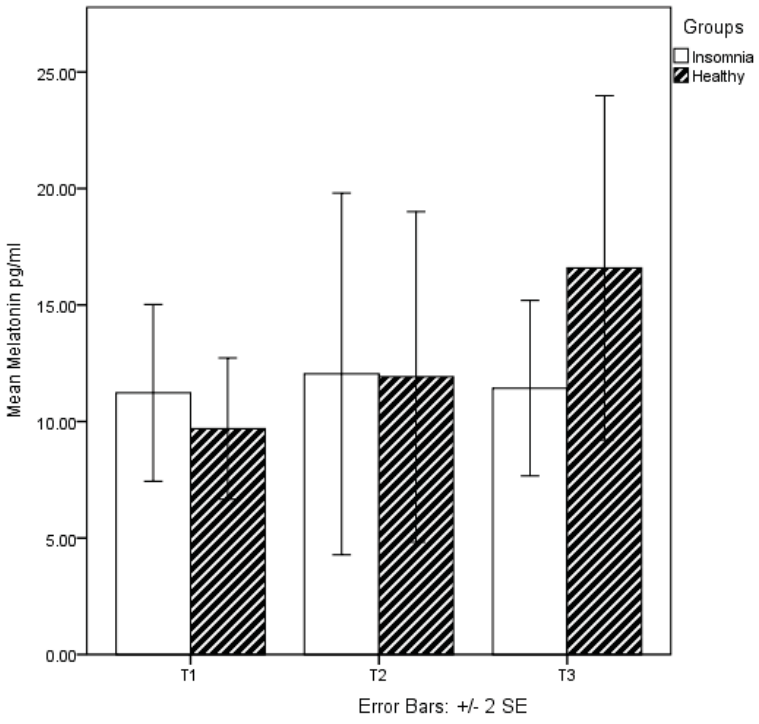

Figure 10: Comparison of melatonin levels in both groups in all trimesters.

Sub-group analysis by one-way ANOVA (Table 3) showed that Disease-match (untreated) insomniac pregnant women have significantly lower melatonin levels (p=0.04) in the third trimester (mean (pg/ml)=8.2±1.33 SEM), when compared to the treated (mean (pg/ml) = 16.8±3.1 SEM) and Healthy pregnant women (mean (pg/ml)=17.4 ±3.5 SEM). No significant difference was found in melatonin levels among the groups in the first

trimester with the following means: Exposed: 8.9±1.6 pg/ml, Disease-match: 9.7±2.0 pg/ml, Healthy: 11.0±1.5 pg/ml. Pairwise comparison showed significant lower melatonin levels in Disease-match compared to healthy group (p=0.03) in the third trimester. Pairwise comparison also found Disease-match group's melatonin levels significantly lower (7.4±2.1 pg/ml) compared to the Exposed group (19.5±6.25 pg/ml) in the second trimester, p=0.03.

Trimesters	Group	N	Mean Melatonin pg/ml	Std. Deviation	Std. Error	P-value
T1	EXP	7	8.9	4.3	1.6	0.7
	DM	13	9.7	7.1	1.9	
	H	18	11.0	6.3	1.5	
T2	EXP	7	19.5	16.5	6.2	
	DM	12	7.4	7.4	2.1	0.2
	H	11	11.9	11.7	3.5	
T3	EXP	5	16.8	6.9	3.1	
	DM	9	8.2	4.0	1.3	**0.04**
	H	12	17.4	12.0	3.4	

Table 3:One-way ANOVA analysis of the three groups in all trimesters. Significant difference observed only in third trimester. EXP: exposed group, DM: Disease-Match, H: Healthy, T1: first trimester, T2: second trimester, T3: third trimester.

Repeated measure analysis was performed for all three groups separately, investigating the pattern of change in melatonin levels in all trimesters as secondary observation. No significant change was found in all groups when all trimesters were compared, p=0.1, p=0.3, p=0.1 for the Exposed, Disease-match, and Healthy groups respectively (Table 4) (Figure 11).

Groups	Trimester	Mean, pg/ml	SD	N	sig.
	T1	10.7	4.1		
EXP	T2	26.0	19.6	4	ns
	T3	18.6	6.4		
	T1	9.3	7.6		
DM	T2	5.8	2.7	9	ns
	T3	8.2	4.0		
	T1	9.7	5.0		
H	T2	11.9	11.7	11	ns
	T3	19.6	19.6		

Table 4: Repeated measure analysis did not show a significant rise of melatonin levels in all trimesters. Exp: exposed, DM: disease-match, H: healthy. ns: non-significant

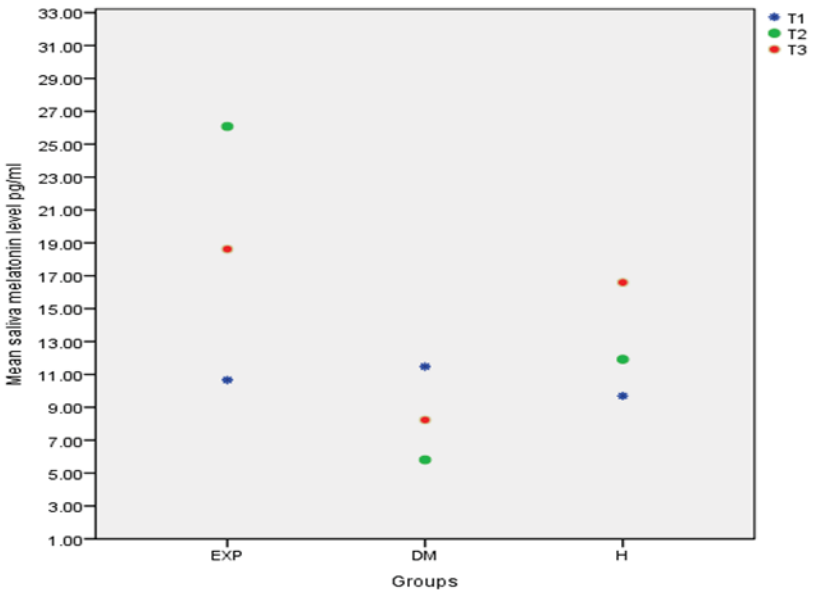

Figure 11: Dot plot of repeated measure analysis of melatonin levels in all trimesters. EXP: exposed, DM: disease-match, H: healthy. T1: first trimester, T2: second trimester, T3: third trimester.

Discussion

Both groups with insomnia were associated with comorbid mental disorders.

Melatonin levels in treated women with psychotropic drugs were similar to healthy controls. A non-significant difference in melatonin levels in insomniac pregnancies (Exposed and Disease- match combined group) compared with Healthy subjects is indicative of higher melatonin level in the exposed group due to the effects of sleep medications. All nine patients in the Exposed (treated) group took medications for either only sleep issues or comorbid symptoms such as depression and anxiety. Five patients took one or combinations of zopiclone, quetiapine, lorazepam, dimenhydrinate, and progesterone as a sleep aid. One patient took a combination of bupropion, amitriptyline, lisdexamphetamine, and clonazepam for insomnia, depression/anxiety and ADD. Trazodone and aripiprazole were taken by another patient for bipolar, mania, and insomnia. Zopiclone, duloxetine and trazodone were used in combination for insomnia and comorbid anxiety. A combination of bupropion and quetiapine was taken for insomnia and comorbid depression.

Amitriptyline decreases sleep latency while treating depression symptoms. It also increases melatonin synthesis by increasing NAS (N-acetyl transferase) in the pineal gland.[84] SSRIs cause increased sleep latency, REM suppression and frequent waking up during the night. As a result, pharmacotherapy addressing the treatment of insomnia should be considered with SSRIs.[85] This is in conflict with a clinical study where duloxetine was shown to increase 6- sulphatoxymelatonin (aMT6s) levels after treatment.[19] Duloxetine was used in one patient for anxiety and trazodone and zopiclone were used concomitantly to improve sleep. Zopiclone has no effect on melatonin concentration when compared to zalplona placebo and a non- benzodiazepine hypnotic in rats.[20] Similar results have been found in healthy volunteers following acute and subchronic administration of zopiclone.[121] A sleep-inducing effect may be due to its hypnotic properties. Increased melatonin levels and improved sleep with trazodone has been documented in clinical studies.[24,25] Bupropion is associated with increased REM (rapid eye movement) sleep,[85] however, a meta-analysis found bupropion was one of the antidepressants that caused insomnia the most often (Figure 12).[86]

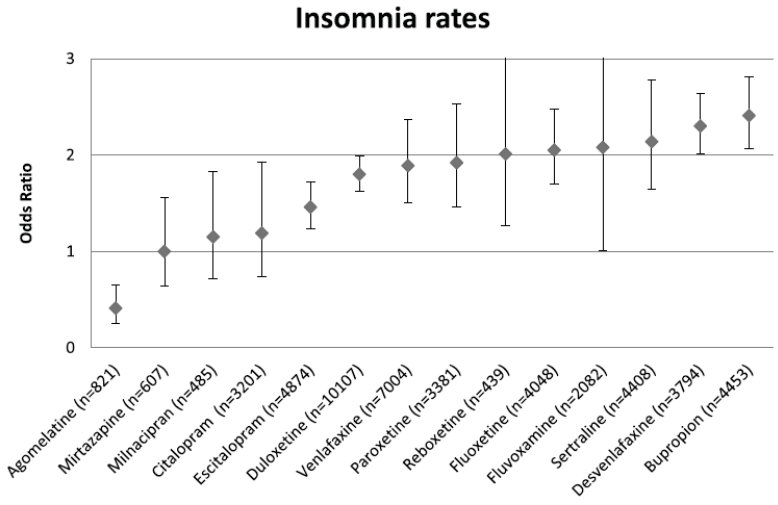

Figure 12: Ascending odds ratios for antidepressants and association with insomnia. Reprinted with permission from Wolters Kluwer Health, Inc.[86]

Low dose quetiapine is shown to have a non-significant effect on melatonin level but significantly reduces nocturnal cortisol levels.[26] A lower cortisol level is associated with a higher melatonin level.[27]

Melatonin levels in women with untreated insomnia have been low throughout pregnancy. This indicates the potential role of melatonin in sleep regulation. Moreover, it further shows the effect of psychotropic medications on sleep and improvement of symptoms which was observed in treated group. Significant lower levels of melatonin observed in the untreated (disease-match) group in the third trimester of pregnancy, compared to the Exposed and Healthy groups, are an indication of the negative role insomnia and sleep disorders can play in modulating melatonin levels.

There were limitations in this study. First and most important was a lack of power due to small sample size. Unmeasured patient compliance was another challenge in this study. Whether patients followed all the instructions they were given from staying in dim light to refrain from food and drugs listed in the instruction sheet were not measured. It was also difficult recruiting pregnant women with insomnia who may also suffer from other comorbid psychiatric conditions. Furthermore, retaining these patients for the duration of pregnancy was another challenge in this study. Therefore, we stopped the study without reaching our optimal calculated sample size.

This study opened the door to future research on the important role of melatonin in the regulation of sleep in pregnancy. More research is needed to confirm the validity of salivary method of melatonin measurement during pregnancy. Assessment of efficacy and reproductive safety of melatonin supplementation in the treatment of insomnia during pregnancy is an important subject of future investigations.

Conclusion

Presently, pregnant women suffering from insomnia are treated with pharmacotherapy, most commonly with sedative hypnotics, benzodiazepines, antihistamines, and antipsychotics. Side effects vary from daytime sleepiness to dependence. Many studies looked at the benefits and risks associated with sleep medications in pregnancy. Fetal risks associated with some of these medications have been confirmed in the studies. Side effects include low risk oral cleft palate, withdrawal symptoms after birth, preterm birth, and low birth weight. [118,188-190] Meanwhile, studies examined the efficacy of the exogenous melatonin in treating sleep disorders. A recent meta-analysis on the efficacy of melatonin for the treatment of insomnia showed a significant decrease in sleep latency, increased sleep time, and a significant positive effect on sleep quality.[118] Results from an open-labeled long-term study showed the efficacy and safety of prolonged-release melatonin (2mg) without any withdrawal symptoms. Results also showed that long term melatonin treatment is not associated

with suppression of endogenous secretion of the hormone as levels measured in urine two weeks after discontinuation. No dependence, relapse of symptoms, or withdrawal symptoms have been reported after discontinuation of treatment.[91] Pilot studies at this time are investigating the role of antenatal melatonin administration as an antioxidant in late pregnancies to prevent or treat IUGR and preeclampsia.[92, 93] The prospect of treating pregnant women with insomnia with the natural sleep hormone melatonin has received very little attention.

This study confirmed the lower saliva levels of melatonin in pregnant women with untreated insomnia. Therefore, melatonin supplementation may be considered an intervention for insomnia in pregnancy. Melatonin is inexpensive, is available over-the counter and it has a low side effect profile. This study confirmed a need for future research to introduce melatonin as a natural hormone therapy for insomnia. The results of this study may have a significant positive effect on the quality of life and health of many pregnant women worldwide currently on sleep medication who have no choice but to continue pharmacotherapy to maintain a stable mental and physical health during pregnancy.

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