University of Pretoria

FACULTY OF HEALTH SCIENCES

DEPARTMENT OF PHYSIOLOGY

The prevalence of sleep apnea in patients with Cushing's syndrome

by

Liechka Groenewald

26097428

Research protocol for the fulfillment of the degree:

MSc Human Physiology

2020

Protocol submitted in fulfillment of the requirements for the degree:

through the Department of Physiology, at the Faculty of Health Sciences, University of Pretoria.

Date: 26/08/2020

Candidate

Name: Liechka Groenewald

Student number: 26097428

Department: Human Physiology

Faculty of Health Sciences

Email: Liechkag@gmail.com

Contact number: 082 970 6040

Supervisor

Name: Prof Peet du Toit

Department: Human physiology

Faculty of Health Sciences

University of Pretoria

Email: peet.dutoit@up.ac.za

Co-Supervisor

Name: Prof A van Schoor

Department: Anatomy

Position: Section of Clinical Anatomy

Senior lecturer

Email: Albert.VanSchoor@up.ac.za

Head of Department

Name: Prof A. Joubert

Department: Human Physiology

Faculty of Health Sciences

University of Pretoria

Co-Supervisor

Name: Ms V. Nortje

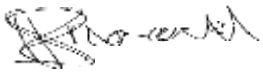
Department: Physiology

Faculty of Health Sciences

University of Pretoria

DECLARATION

I, Liechka Groenewald, hereby declare that this research dissertation is my own work and had not been presented for any degree at another University.



Signed:…….

Date: ……26-08-2020…….

Department of Physiology, School of Medicine, Faculty of Health Sciences, University of Pretoria, South Africa.

ACKNOWLEDGEMENTS

It has been an absolute pleasure to be entrusted with this project and to contribute to the ever-growing field of medical research. It is my privilege and honour to acknowledge the following people for contributing to the completion of this dissertation.

My supervisor, Prof PJ du Toit, thank you for all your patience with me. Thank you for all your time, leadership and guidance with this project.

My co-supervisor, Prof A van Schoor, thank you for always replying extremely quickly and for always being willing to help.

To Charl Janse van Rensburg, thank you for helping regarding the statistical analysis.

Dr N.P. Ranchhod, thank you for your encouragement and all your patience with me and always motivating me. Thank you for your assistance with my data collection and by collaborating with the other doctors.

Thank you to my family and friends who was always willing to listen when I wanted to give up and who advised and motivated me throughout this journey.

Abstract

Patients with Cushing's syndrome often complain about sleep disruption and excessive day time sleepiness, which could contribute to worsening quality of life and metabolic comorbidities (obesity, hypertension, diabetes mellitus, dyslipidaemia) associated with hypercortisolism. Sleep disorders have been shown to increase the risk of developing cardiovascular disease and that the risk of cardiovascular disease in patients with hypercortisolism may be worsened by impaired sleep. Cushing's syndrome patients may also be at increased risk for obstructive sleep apnea due to their obesity.

Symptoms of disordered sleep are common in the general global population, with early morning awakenings and middle-night awakenings observed in over 50% of patients. Increasing evidence has shown a significant relationship between Cushing's syndrome and the risk of developing obstructive sleep apnea.

Only a few studies with a small cohort of patients have evaluated sleep disorders in Cushing's syndrome using polysomnography. This study was the first in determining the prevalence of obstructive sleep apnea and sleep alterations in patients with Cushing's syndrome in a South African setting and these findings were compared with those of an age-matched group of healthy control subjects.

This study determined the prevalence of obstructive sleep apnea in patients with Cushing's syndrome. Sleep studies that were performed from May 2017 until September 2019 were used in this study. To compile a more detailed study, the study had 3 groups. These values were correlated with the normative values found in the literature. Group one had normal polysomnograms.

The second group were the patients without Cushing's syndrome who were diagnosed with sleep apnea. This group was used to determine the prevalence of sleep apnea in non-Cushing's syndrome patients.

The last group consisted of patients who were diagnosed with Cushing's syndrome. The prevalence of sleep apnea in Cushing's syndrome patients were compared to the previous two groups.

In this study, sleep apnea was observed in 68% of patients with Cushing's syndrome; this number is higher compared to other similar studies. However out of the total study population 74% of patients had sleep apnea, which is higher than the global prevalence of OSA.

Keywords: Obstructive sleep apnea (OSA), Sleep apnea, Hypopnea, Apnea, Apnea/hypopnea Index (AHI), Cushing's syndrome

Table of Contents:

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

5- HT	5-Hydroxytryptamine
AASM	American Academy of Sleep Medicine
ACh	Acetylcholine
ACTH	Adrenocorticotrophic hormone
AHI	Apnea/ hypopnea index
ARAS	Ascending Reticular Activating System
BF	Basal forebrain
BMI	Body mass index
CNS	Central nervous system
CPAP	Continuous positive airway pressure
CRH	Corticotropin releasing hormone
CSA	Central sleep apnea
CT	Computed tomography
DA	Dopaminergic neurons
DDAVP	Desmopressin
ECG	Electrocardiogram
EDS	Excessive daytime sleepiness
EEG	Electroencephalogram / Electroencephalographic
EMG	Electromyographic
EOG	Electro-oculogram
FDA	Food and drug administration
GABA	γ-aminobutyric acid/ Gamma-Aminobutyric acid
GAL	Galanin
GH	Growth hormone
His	Histaminergic neurons
HPA	Hypothalamus-pituitary-adrenal
IPSS	Inferior petrosal sinus sampling
LC	Locus Coeruleus
LDT	Laterodorsal tegmental
MCH	Melanin-concentrating hormone
MRI	Magnetic resonance imaging
MT	Movement time

NE	Norepinephrine
NREM	Non-rapid eye movement
NTS	Nucleus stactus solitarius
ORX	Orexin
OSA	Obstructive sleep apnea
PDR	Posterior dominant rhythm
POSTS	Positive occipital sharp transients of sleep.
PPT	Pendunculopontine tegmental
P-REM	Phasic REM
PSG	Polysomnography
RAS	Reticular activating system
REM	Rapid eye movement
SA	Sleep apnea
SMR	Standard mortality ratio
SREM	Slow rolling eye movements
SWS	Slow wave sleep
TMN	Tuberomammillary nucleus
T-REM	Tonic REM
TSS	Trans-sphenoidal selective
TST	Total sleep time
VLPO	Ventrolateral preoptic
vPAG	Ventral periaqueductal gray matter

List of Appendixes

CHAPTER 1: INTRODUCTION

Sleep is defined as unconsciousness from which a person can be aroused by sensory or other stimuli[1]. It is therefore a reversable state of reduced responsiveness[2]. The normal physiology of sleep is subject to sleep drivers and follows a specific architecture that includes both a non-rapid eye movement (NREM) component and a rapid eye movement (REM) component[3]. The two states of sleep alternate cyclically during the night[4]. Sleep phases can be differentiated by means of an electroencephalogram (EEG)[2-3]. The EEG shows different patterns of brain waves during each sleep phase[1].

The different types of sleep have different qualities. NREM sleep is exceedingly restful and is associated with decreases in both peripheral vascular tone and many other vegetative functions of the body such as a reduction in blood pressure, respiratory rate and basal metabolic rate[1]. During NREM sleep there is also cardiovascular stability, growth hormone secretion and a global drop in blood flow[3]. REM sleep on the other hand is not so restful, and it is often associated with vivid dreaming[1] and an increase in blood flow to the brain and increased brain metabolism[3]. Although the brain is highly active during REM sleep, the body's motor functions are inhibited[3].

Sleep begins with NREM sleep, the first REM sleep cycle only occurs 80-100 minutes later. Thereafter NREM and REM sleep cycles occurs with a period of 90 minutes. REM sleep episodes lengthen across the night[4].

Sleep disorders have an impact on the structure and distribution of sleep[4]. Obstructive sleep apnea (OSA), which is the most common form of sleep-disordered breathing, is a clinical condition characterized by recurrent episodes of complete obstruction (apnea) or partial obstruction (hypopnea) of the upper airway during sleep[5-6].

Sleep apnea syndrome causes fragmentation of sleep and can increase the frequency of arousals. Sleep apnea is also associated with suppression of slow wave sleep (SWS) or REM sleep secondary to the sleep-related breathing problem[4].

OSA is the most common sleep disorder and causes sleep fragmentation, intermittent hypoxia during sleep[5], daytime somnolence, neurocognitive defects, and depression[6]. It affects almost every system in the body, resulting in an increased incidence of

hypertension, cardiovascular disease, stroke, pulmonary hypertension, cardiac arrhythmias, obesity and altered immune function. It also increases the risk of having an accident, presumably as a result of associated somnolence[6].

Sleep related signs of OSA are usually observed by the bed partner, such as loud snoring alternating with periods of silence associated with paradoxical movements of the chest and abdomen. These periods of silence are terminated by a loud gasp, choking or snort[4].

Excessive daytime sleepiness and the tendency to fall asleep in inappropriate situations as well as a lack of energy occurs. Other common symptoms of OSA includes awakening with a dry mouth, which may reflect mouth breathing, restless sleep and nocturnal diaphoresis. Morning headaches occur often in patients with OSA. The headaches usually resolves within 30 minutes[4].

Nocturia is another common symptom of patients with sleep apnea and is also associated with disturbed sleep with complaints of daytime somnolence. Nocturnal gastroesophageal reflux are also complains reported by patients with OSA[4].

There is an increase in mortality due to sleep disturbances caused by OSA and is likely to account for much of the association between sleep complaints and adverse outcomes[4]. Sleep-disordered breathing is sadly underdiagnosed and undertreated[4]. The prevalence of sleep apnea increases with increasing age and symptoms includes sleepiness, hypertension and cognitive dysfunction[4]. It is likely that the underdiagnosis of sleep apnea in older people is even more common than in younger people[7]. The “classic” clinical presentation of OSA is uncommon in older adults, which may account in part for the reduced prevalence of clinical diagnosis of the disorder in this population. With aging, loss of tissue elasticity also may contribute to airway collapse[4].

Sleep apnea has been found to be more common in patients with endocrine disorders such as Cushing’s disease and Cushing’s syndrome[8]. Patients with Cushing’s syndrome may have more sleep disturbances such as insomnia, difficulty falling asleep or waking during the night or in the early morning[9], fatigue and a multitude of psychiatric syndromes, including frank psychosis and major depression[8]. Most OSA patients complain of daytime fatigue/ sleepiness [9-10]. There is a significant relationship between Cushing’s syndrome and other risk factors such as OSA, obesity, diabetes,

dyslipidaemia and hypertension[9]. Cushing's syndrome and OSA have rarely been investigated and was first described in 1992 [9].

Cushing syndrome is due to the chronic, excessive and inappropriate exposure to glucocorticoids[11-12]. This is known as endogenous Cushing's syndrome. The most common cause of Cushing's syndrome is the use of exogenous glucocorticoids, frequently needed to treat inflammatory conditions[11]. Presenting features commonly include weight gain, growth retardation, hirsutism, obesity, striae, acne and hypertension[13].

The clinical "Cushingoid" phenotype is well recognized and is due to the long-term adverse effects of excess glucocorticoid and may be indistinguishable from endogenous Cushing's syndrome on clinical grounds alone[11]. The diagnosis cannot be made unless both clinical features and biochemical abnormalities are present[12].

Diagnostic approaches are based on distinguishing between adrenocorticotropic hormone (ACTH)-dependent and ACTH-independent aetiologies[12-13]. The ACTH-dependent forms are characterized by excessive ACTH production from a corticotroph adenoma (known as pituitary-dependent Cushing's syndrome or Cushing's disease), from an ectopic tumoral source (ectopic ACTH syndrome), or from normal corticotrophs under the influence of excessive corticotropin-releasing hormone (CRH) production (ectopic CRH secretion)[12].

Increasing evidence has shown a significant relationship between Cushing's syndrome and risk factors for OSA such as obesity, hypertension, diabetes, dyslipidaemia[14-15]. However, the association of Cushing's syndrome with OSA itself, has rarely been investigated and data regarding the association between Cushing's syndrome and OSA is scarce.

The purpose of the research is to demonstrate that patients with Cushing's syndrome have an elevated risk of developing OSA (Figure 1: Methodology).

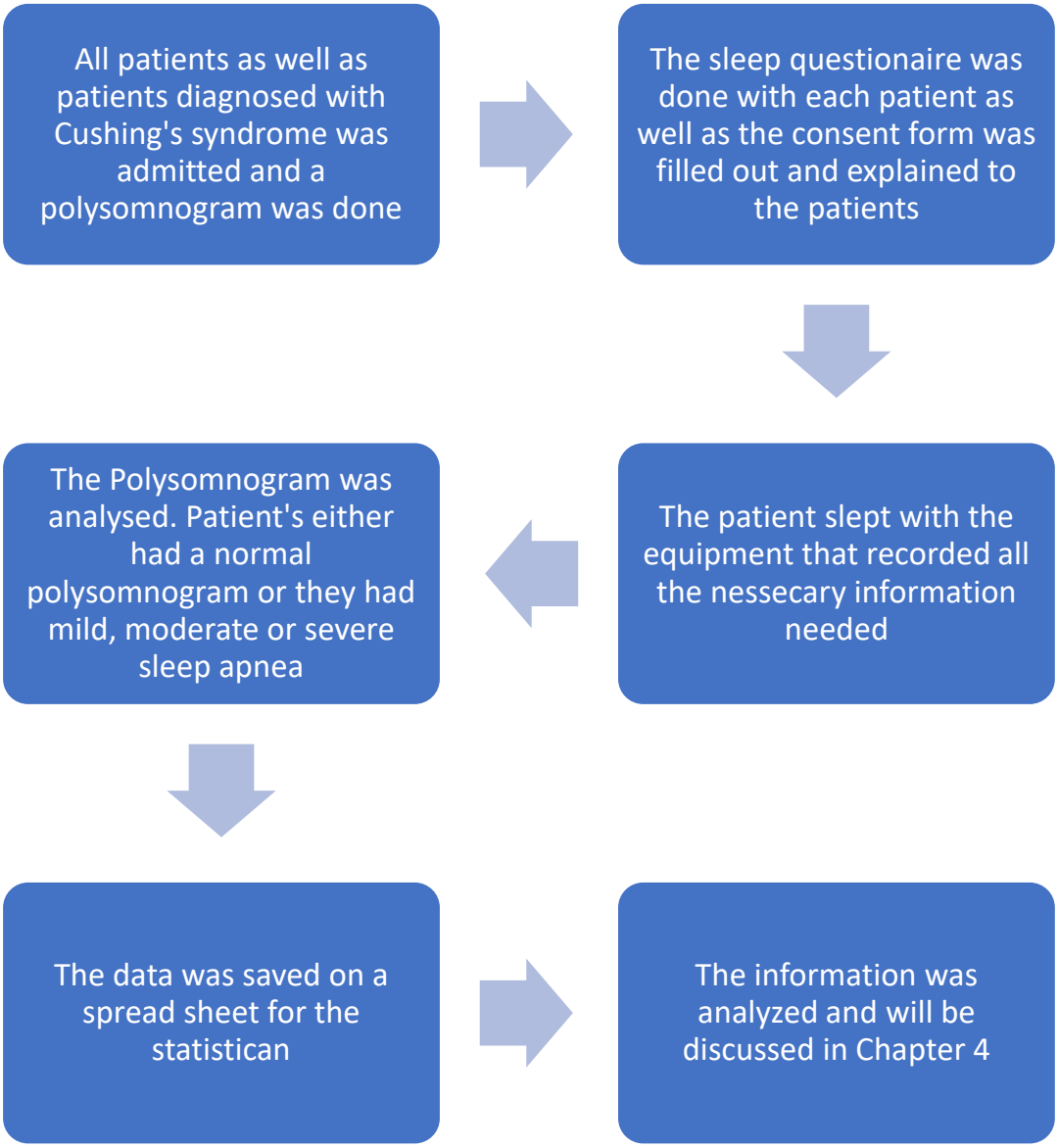


Figure 1: Overview of the contents of this study.

CHAPTER 2 – LITERATURE REVIEW

Sleep Physiology

Humans spend approximately one-third of their lives asleep, yet few people know much about sleep[16]. Scientific research in the area of sleep and its disorders has also been slow and its functions remains to be fully elucidated. Sleep is a universal need of all higher forms of life including humans and its absence has serious physiological consequences[16-18].

Sleep is a behavioural state of reduced responsiveness and perceptual separation from the environment[4]. In 1830 Macnish[17] defined sleep as “suspension of sensorial power” in which the voluntary functions are absent but the involuntary functions, such as circulation, respiration, and other functions controlled by the autonomic nervous system remain intact[17].

During sleep there is also reduced motor activity as well as reduced metabolism[2]. This is a reversible state. Sleep is a complex combination of physiologic and behavioural processes[4]. The behavioural criteria include reduced mobility, closed eyes, reduced response to external stimuli, quiescence, impaired cognitive function, and a reversible state of unconsciousness. Physiological criteria are based on findings from electroencephalography, electro-oculography, and electromyography, as well as physiological changes in cardiovascular and respiratory function[17].

In 1928 a German psychiatrist, Hans Berger, recorded the electrical activity of the human brain[19]. He was able to demonstrate differences in rhythms between subjects who were asleep versus subjects who were awake. Berger correctly inferred that the signals he recorded were of brain origin and called these signals electroencephalograms. For the first time, by using his method, the occurrence of sleep could be established without disturbing the sleeper. As a result, sleep could be measured continuously[19].

During the course of 1937 – 1938 brain wave patterns were described by Loomis, Harvey, Hobart, Davis, and others at Harvard University[4]. Alfred Lee Loomis played a pivotal role in developing amplifier systems to record sleep. He identified a sleep

feature consisting of high voltage waves seen sporadically in sleep and these discharges were named “K-complexes”[4].

In the meantime, at the University of Chicago, Blake, Gerard, and Kleitmann added to the work from their studies on the human EEG[4]. They characterized sleep by high-amplitude slow waves and spindles. Whereas wakefulness was characterized by low-amplitude waves and alpha rhythm. In the 1930’s until recently, the brain wave recordings were done with paper and ink recordings and recently digital recordings exist[4].

Sleep Architecture

There are two states of sleep. Firstly, NREM sleep and REM sleep[2,4]. To differentiate between the different stages of sleep, EEG measurements of the brain activity, as well as electromyographic (EMG) measurements of muscle activity are monitored [2]. These two states alternate cyclically across a sleep episode (Figure 2). There are well defined variabilities between the two states. NREM sleep includes a variably synchronous cortical EEG. This includes features of sleep spindles, K-complexes and slow wave activity. Slow wave activity that occurs is theta (4-7Hz) and delta activity (1-3Hz). NREM is also associated with low muscle tonus and minimal psychological activity. During REM sleep the EEG is desynchronized, muscles are atonic and dreaming occurs[4].

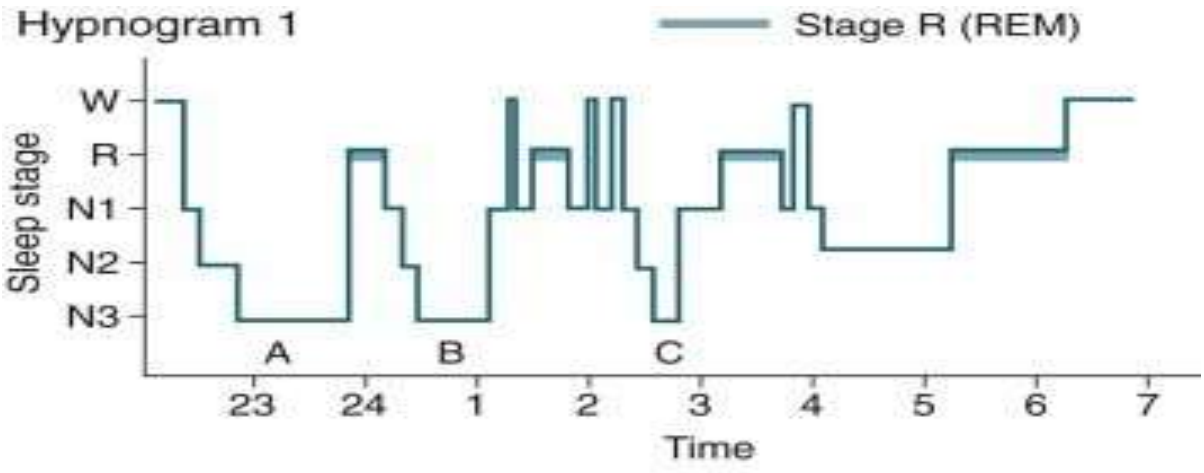


Figure 2: A hypnogram of normal sleep stages throughout the night. W (Wakefulness, N1 (Stage 1), N2 (Stage 2), N3 (Stage 3) and R (REM sleep)[20].

During wakefulness when the patient's eyes are closed, one will see the posterior dominant rhythm (PDR). This rhythm, in a normal awake patient, will consists of alpha activity. Alpha activity occurs between 8-12Hz (Figure 3). The waveform of alpha is rhythmic and sinusoidal and has the highest voltage in the posterior or occipital regions. It is less prominent in the anterior regions[21-22]. The PDR is seen when the patient's eyes are closed and it attenuates/disappears when the patients open their eyes (Figure 4). The rhythm returns when the eyes are closed again[21].

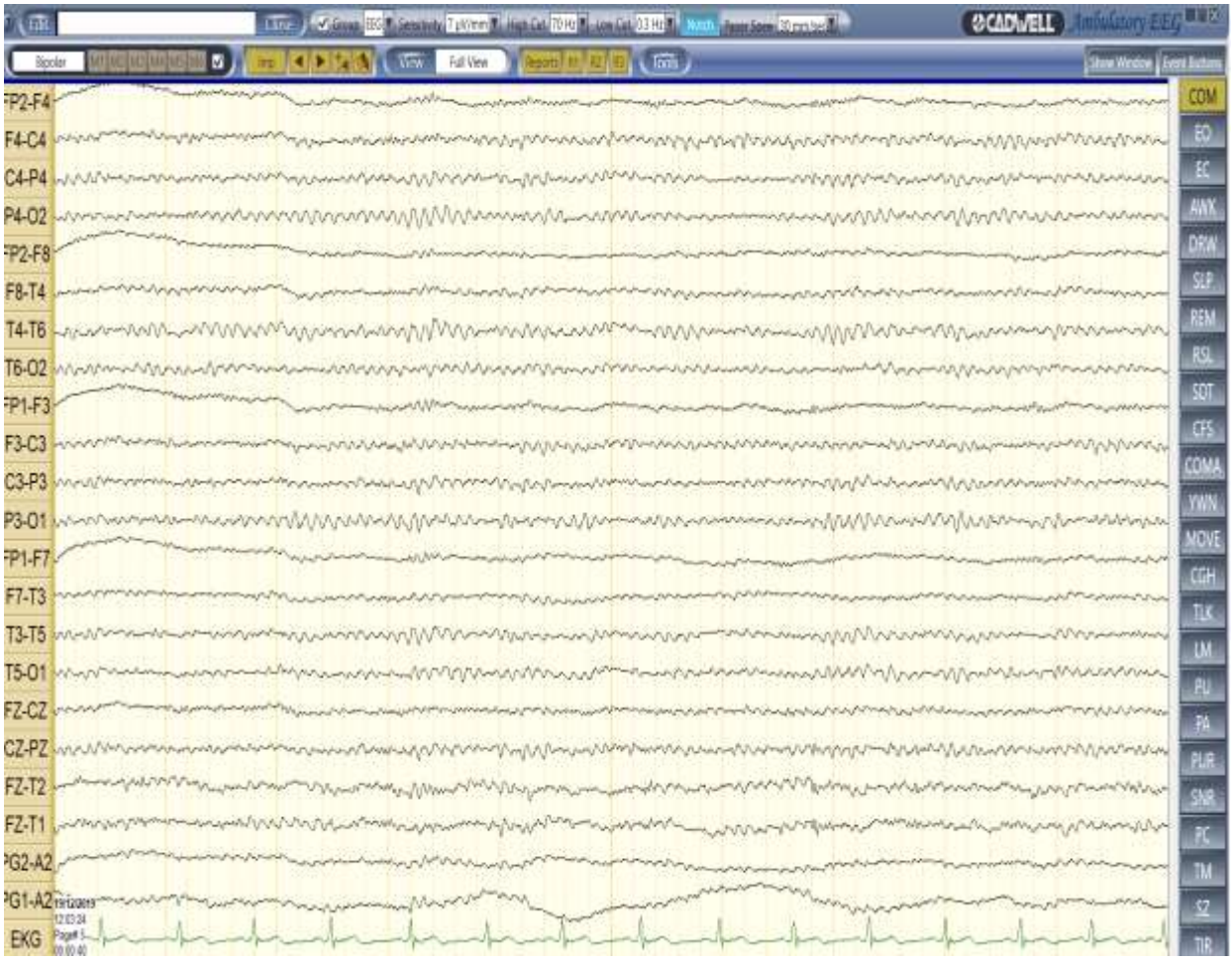


Figure 3: EEG example of a normal awake patient with eyes closed. Background PDR is in the alpha frequency range with beta activity. Adapted by Liechka Groenewald.

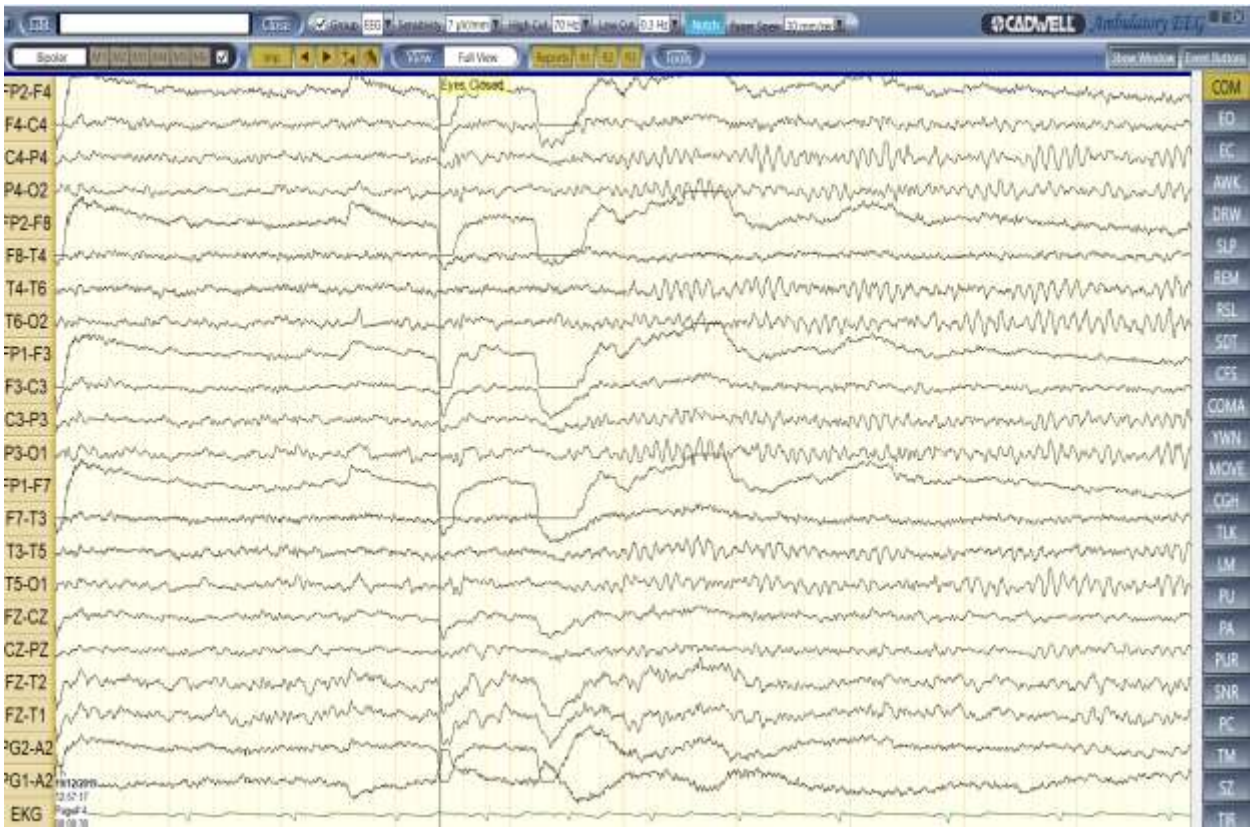


Figure 4: A normal example of alpha activity that attenuates during eye opening and returns during eye closure. Adapted by Liechka Groenewald.

The transition from wakefulness to drowsiness shows gradual alpha dropout and diffuse low voltage slow activity[22]. The period of PDR slowing is not always identifiable and simply drops out without an observable period of slowing. Slow roving lateral eye movements are another finding in drowsiness (Figure 5). These slow roving eye movements are detected by the additional electro-oculogram (EOG) electrodes, but are not visible on casual observation of the patient[21]. With deepening drowsiness vertex waves appear[21-22] (Figure 6). Vertex waves may appear as burst and will continue in a repetitive fashion through stage II sleep[21]. Positive occipital sharp transients of sleep (POSTS) may be present[22] as shown is Figure 7.

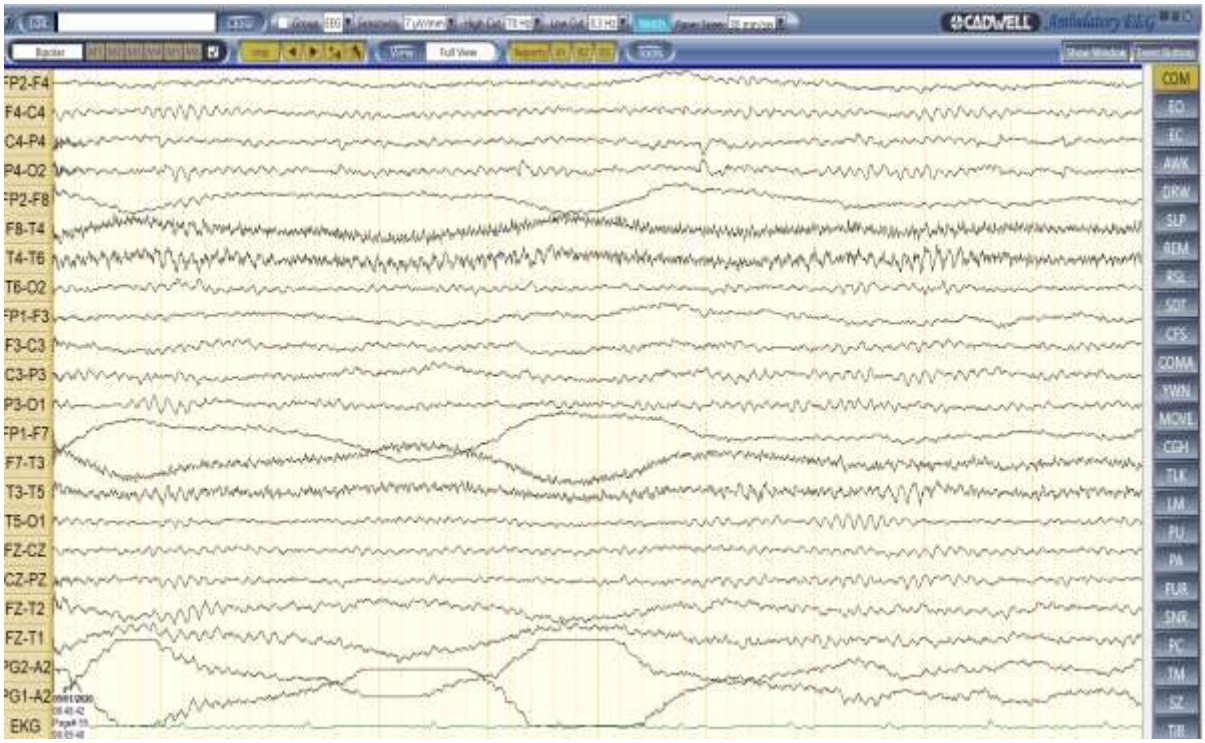


Figure 5: Normal slow roving eye movements during drowsiness. Adapted by Liechka Groenewald.

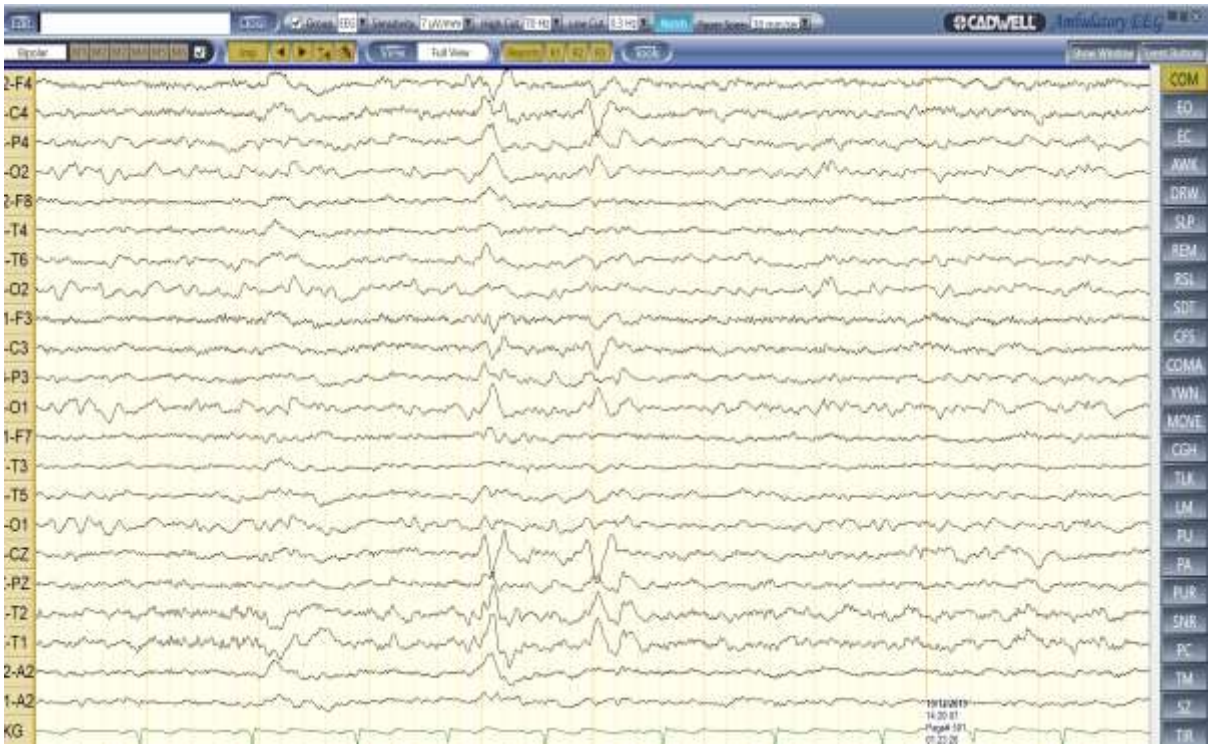


Figure 6: Features of vertex waves during sleep. Adapted by Liechka Groenewald

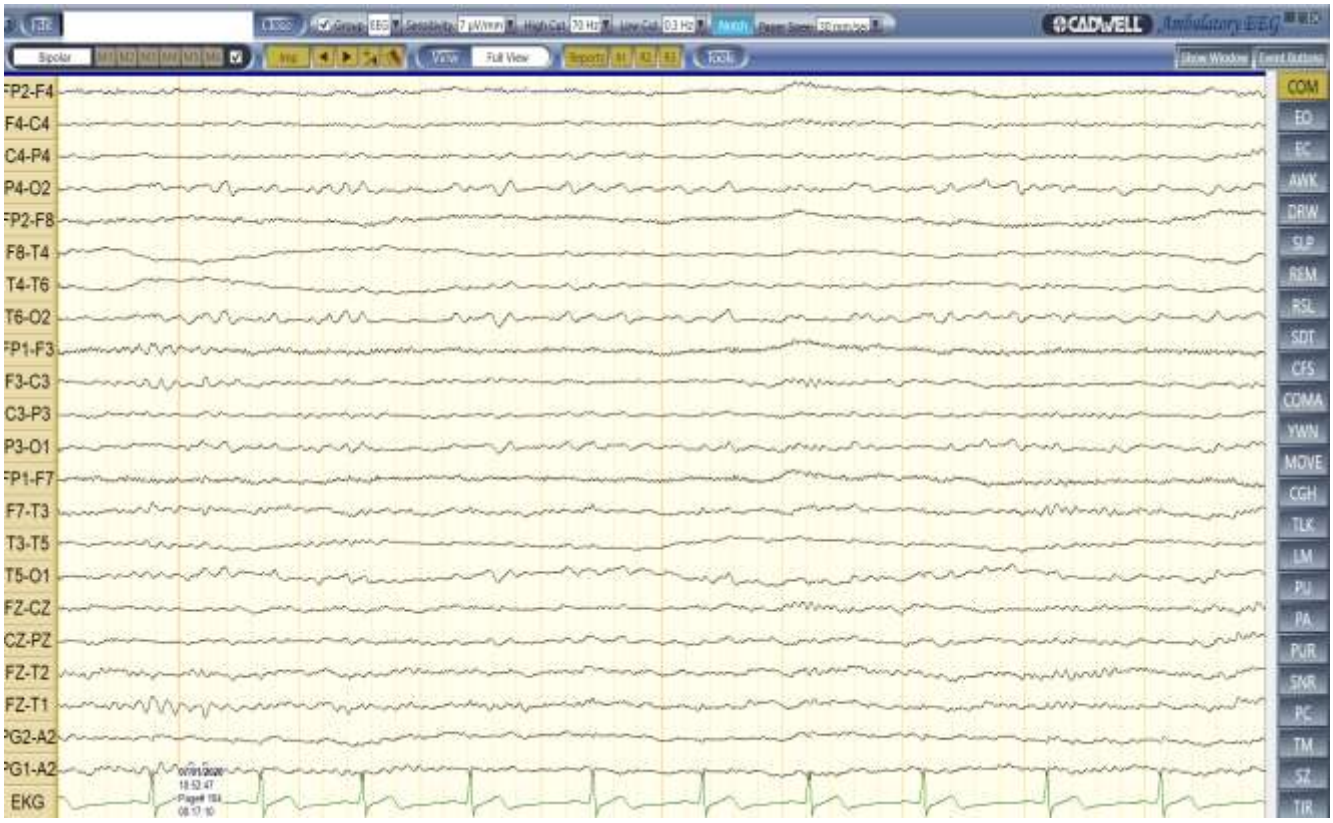


Figure 7: Features of POSTS in the occipital regions during drowsiness and light sleep. Adapted by Liechka Groenewald

Transition to stage II sleep is characterized by the appearance of sleep spindles, K-complexes and less than 20% of the epoch containing delta waves. Figure 8 and Figure 9 show examples of sleep spindles and K-complexes occurring in an EEG. Sleep spindles consists of 11-15 cycles per second central bursts[22]. Sleep spindles lasts from 1 second to 3 seconds. In deeper stage II sleep, the field of sleep spindles may include both the frontal and central areas. Bursts of high voltage waves occurring across nearly all channels may be seen sporadically in sleep. These discharges are called K-complexes and can be mistaken for spike-wave discharges[21]. K-complexes consists of all or any 2 of the 3 main components, i.e. a negative vertex sharp-wave maximum at the midline central electrodes, a following negative slow wave maximal frontally and a sleep spindle maximal in the central regions. They must be at least 0.5 seconds in duration before they can be scored[22].

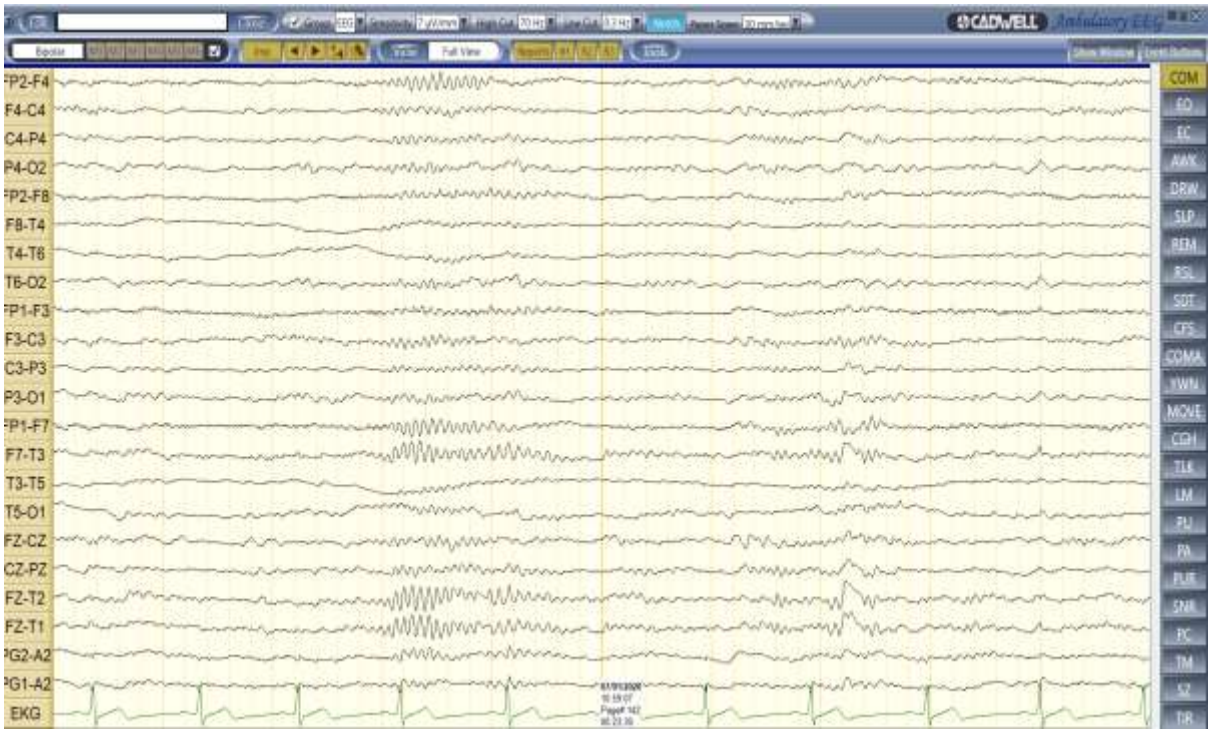


Figure 8: Features of sleep spindles during stage II sleep. Adapted by Liechka Groenewald

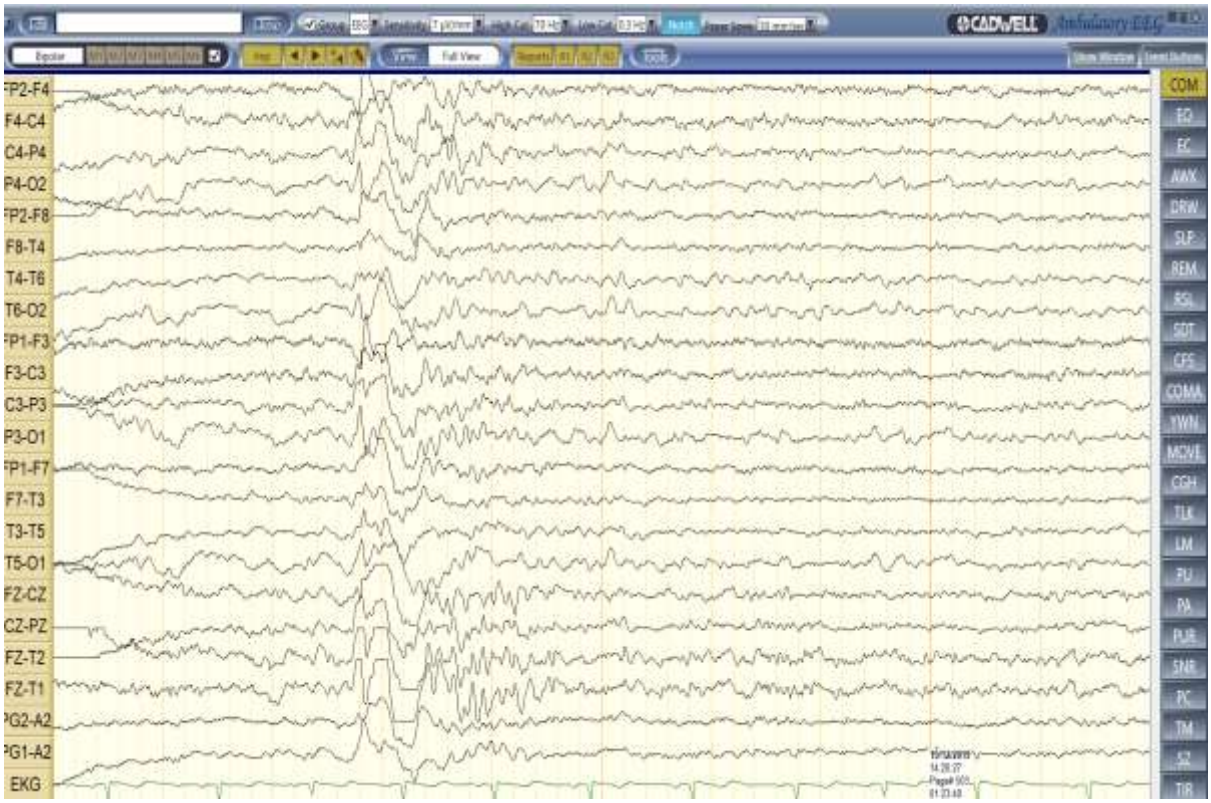


Figure 9: Normal features of a K-complex during stage II sleep. Adapted by Liechka Groenewald

Stage III sleep is scored when the background activity contains higher voltage delta waves of 0.5-3 cycles per second. Sleep spindles can still be present, but will gradually become less[22] (Figure 10).

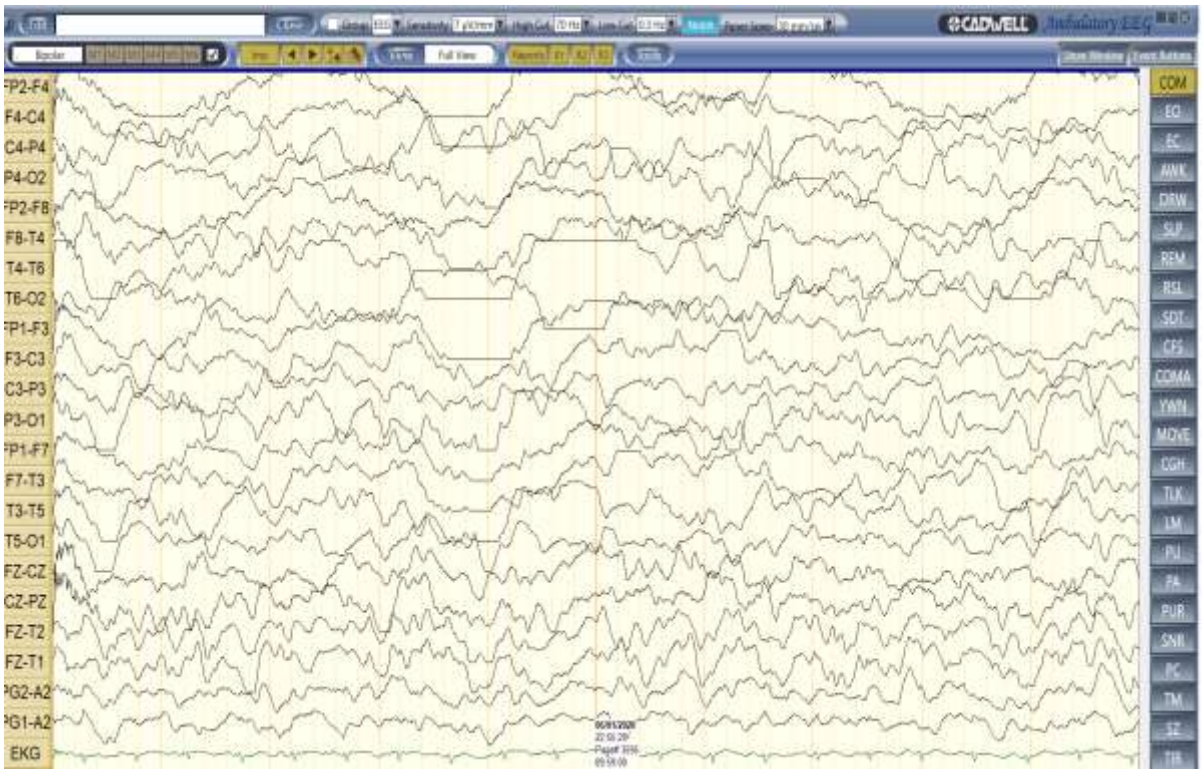


Figure 10: High voltage delta activity occurring during stage III sleep. Adapted by Liechka Groenewald

REM sleep contains low voltage EEG activity and is similar to stage I sleep, however patterns associated with rapid eye movements either in bursts or in isolation. An example of REM sleep is shown in Figure 11. There is general absence of sustained axial musical tone in the submental EMG. Bursts of sawtooth waves may occur. They will occur generally before a REM burst[22].

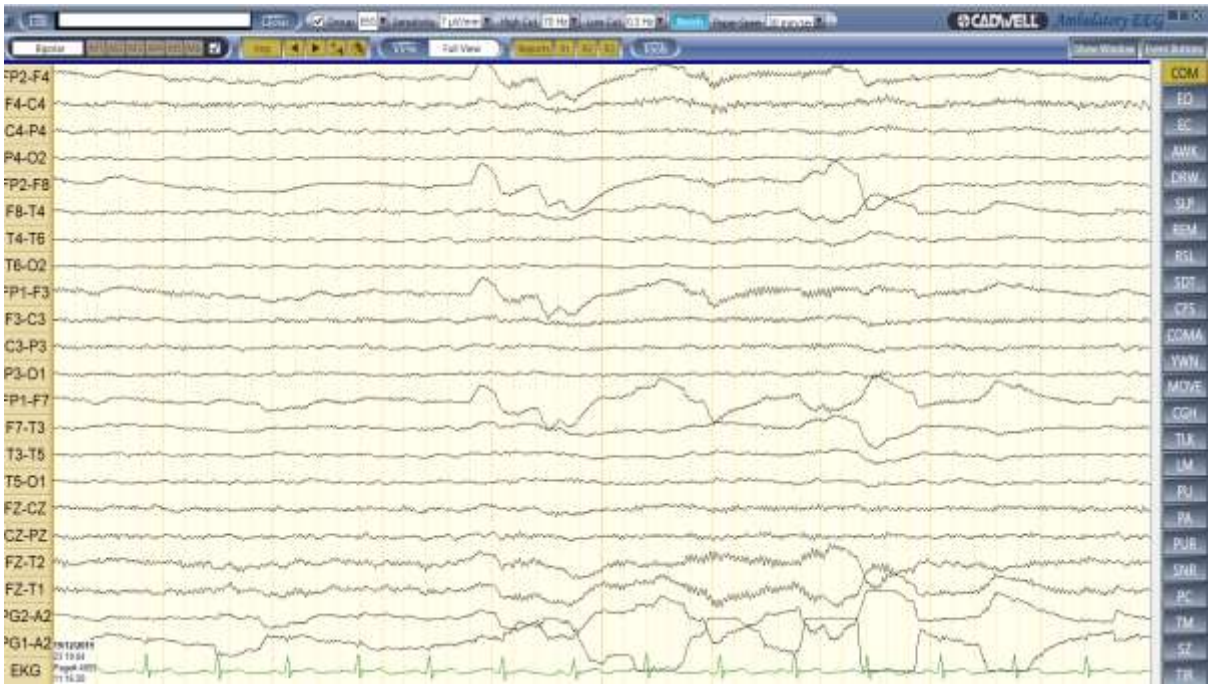


Figure 11: REM sleep with rapid eye movement activity. Adapted by Liechka Groenewald

Arousal from sleep is a quick process with almost immediate change from sleep into an awaking pattern[22]. It may occur uneventfully with the return of the PDR and other patterns of wakefulness. Other times arousal can be marked by a run of diffuse, high voltage rhythmic waves called an arousal hypersynchrony[21].

As shown in table 1, NREM is subdivided into three stages, stage 1, 2 and 3. The arousal threshold is generally lowest in stage 1 and highest in stage 3. NREM sleep is associated with minimal or fragmentary mental activity. REM sleep is defined by EEG activation, muscle atonia and episodic bursts of REM. REM sleep isn't divided into different stages, however for certain research purposes tonic and phasic types of REM are of interest. During REM sleep the mental activity is associated with dreaming. Most people are able to recall a dream after being aroused from this state of sleep [4].

The sleep pattern begins with NREM sleep and progress through deeper stages of sleep. Approximately 80 to 100 minutes after sleep onset the first REM cycle will occur (Figure 2). Thereafter, NREM and REM sleep cycles within a period of 90 minutes occurs. During the early NREM cycles, the SWS will occur more frequently and REM sleep will lengthen across the night [4].

Sleep microstructure includes momentary dynamic episodes, such as arousals. An arousal is a shift in EEG frequency lasting for 3-14 seconds and includes alpha, beta or theta activities but not spindles or delta waves. The person must be asleep for 10 consecutive seconds before an arousal can be scored. The microstructure also includes K-complexes and sleep spindles[17].

Table1. Sleep-stage electroencephalographic (EEG) rhythms and characteristics summarize sleep-stage scoring rules according to both the traditional Rechtschaffen and Kales system and the current system developed by the American Academy of Sleep Medicine. MT, movement time; REM, rapid eye movement; P-REM, phasic REM; SREM, slow-rolling eye movements; T-REM, tonic REM; TST, total sleep time [19].

Properties	**Stage Wake(W)**	**N1 (Stage1)**	**N2 (Stage 2)**	**N3 (Stage 3 and 4)**	**REM sleep**
EEG	Eyes open: Low voltage, mixed frequency. Alpha attenuates. Eyes closed: Low voltage, high frequency. More than 50% alpha activity.	Low voltage, mixed frequency. Theta activity and vertex sharp waves.	Low voltage, mixed frequency. At least one K-complex/sleep spindle.	Stage 3 is up to 20-50% high amplitude delta activity. Stage 4 is more than 50% high amplitude delta activity.	Low voltage mixed frequency. The presence of sawtooth waves. Desynchronised EEG.
EOG	Eye blinks, voluntary control, slow rolling eye movements when drowsy.	Slow rolling eye movements.	Occasional slow rolling eye movements.	Mirrors EEG.	Phasic REM.
EMG	Tonic activity, high EMG activity. Under voluntary control.	Tonic activity. High-medium EMG activity.	Tonic activity, low EMG activity.	Tonic activity, low EMG activity.	T-REM: Relatively reduced. P-REM: episodic EMG twitching.

Duration		10 minutes	20 minutes	30-45 minutes	The first REM period is very short, lasting about 5 minutes, the second episode lasts about 10 minutes and the third is roughly 15 minutes. The final REM period usually lasts for 30 minutes and sometimes up to an hour.
Properties	**Stage Wake(W)**	**N1 (Stage1)**	**N2 (Stage 2)**	**N3 (Stage 3 and 4)**	**REM sleep**
Arousal Threshold		Lower	Lower	Highest	Low
Physiologic changes		Progressive reduction of physiologic activity, blood pressure, heart rate slows down			T-REM: Muscle paralysis, increase cerebral blood flow. P-REM: Irregular breathing, variant heart rate, phasic muscle twitch
%Total Sleep Time	2-5%	10-15%	44-55%	3-8%	20-25%
Dreaming				Diffuse dreams	Vivid, bizarre and detailed dreams.

Parasomnias & movement disorders	Hypnic jerks in transition to N1	Hypnic jerks	Confusional arousal somniloquy	Sleep walking, night terrors	REM sleep behaviour disorder, REM nightmares

Physiology during Sleep

A number of physiological changes occur during NREM and REM sleep that are different from those noted during wakefulness[17]. Sleep has the ability to reduce body activities as well as brain metabolism while still allowing a high level of responsiveness. The following physiological changes occur in the systems below:

Cardiovascular: Changes in the heart rate, blood pressure, cardiac output and the peripheral vascular resistance decreases during NREM sleep[16]. These functions decrease even more in REM sleep. This is primarily regulated by the autonomic nervous system. During NREM sleep the cerebral blood flow and the cerebral metabolic rate for glucose and oxygen decreases between 5%-23%. These values increase by 10%-41% above waking levels during REM sleep. This indirectly suggests that NREM sleep is the state of resting brain, with reduced neuronal activity, decreased synaptic transmission and depressed cerebral metabolism. This would also suggest that REM sleep represents an active brain state with an increase in neural activity and increase in brain metabolism[17].

Because of all the hemodynamic and sympathetic alterations, REM sleep, which is prominent during the third part of the night's sleep, could initiate increased platelet aggregation, plaque rupture, and coronary artery spasm. These increases may act as triggering mechanisms for thrombotic events causing myocardial infarction, ventricular arrhythmias, or even sudden cardiac death[17].

Somatic central nervous system: The firing rates of many neurons in the central nervous system (CNS) decreases during NREM sleep and increases during REM sleep[17].

Autonomic nervous system: Autonomic changes occurring in sleep involves respiration, circulation and thermoregulation. During NREM sleep there is an overall

decrease in the sympathetic activity and tonic increase in parasympathetic activity. This activity increases even more during tonic REM sleep. During phasic tonic REM sleep, sympathetic activity decreases. During phasic REM sleep the sympathetic activity increases intermittently[17].

Cerebral blood flow: NREM sleep is associated with a reduction in blood flow and metabolism, while total blood flow and metabolism in REM sleep is comparable to being awake[16].

Thermoregulation: Body temperature has been linked intimately to the sleep-wake cycle, but it follows a circadian rhythm that is independent of the sleep-wake rhythm. A person's body temperature begins to fall at the onset of sleep and reaches its lowest point during the third sleep cycle. Thermosensitive neurons show and increase in firing rates at sleep onset causing peripheral vasodilation, heat loss, fall of body temperature and increased EEG slow wave activity. Thermoregulation is maintained during NREM sleep but is non-existent in REM sleep. Thus, physiological responses (e.g., shivering, panting, sweating, and piloerection) to thermal stimuli are depressed or absent during REM sleep[17].

Gastrointestinal changes: There are variable gastric acid responses during sleep in normal individuals. During sleep swallowing is suppressed, and there is prolonged acid clearance. These factors are important in the pathogenesis of esophagitis caused by nocturnal gastroesophageal reflux. Esophageal motility is reduced during sleep[17].

Endocrine: Profound changes in the neuroendocrine secretions are found during sleep[17]. Growth hormone, thyroid hormone and melatonin secretion are influenced by sleep[16]. Secretion of growth hormone exhibits a pulsatile increase and typically takes place during the first few hours after sleep onset and occurs during SWS. Thyroid hormone secretion reaches a peak in the evening and then decreases throughout the night. Melatonin, which is synthesized and released by the pineal gland and derived from serotonin is responsible for sleep induction by reducing the alerting effect from the suprachiasmatic nucleus. Melatonin begins to rise in the evening and decreases to low levels during the day. It is influenced by the light-dark cycle and is inhibited by light[16-17].

In humans the amount of cortisol present in the blood undergoes diurnal variation [23]. Cortisol levels peak in the early mornings (8am) and reaches its lowest levels at midnight - 4am, or 3-5 hours after the onset of sleep. This pattern is not present at birth, estimates of when it begins vary from 2 weeks to nine months of age[24].

Respiratory: There are two systems that control respiration during sleep and wakefulness. One is metabolic or autonomic and the second is voluntary or behavioural control. Metabolic and voluntary systems functions during wakefulness, but only the metabolic system operates during NREM sleep. The wakefulness stimuli that act through the ascending reticular activity system (ARAS) also act as tonic stimuli to ventilation. The activity decreases in the respiratory neurons in the medulla[17].

During NREM sleep the respiratory muscle activity decreases slightly, but during REM sleep is decreased markedly. A marked decrement or even temporary suppression of intercostal muscle tone occurs during REM sleep, whereas tonic activity of the diaphragm diminishes and phasic activity continues[16]. Muscle tone in the upper airway decreases in NREM sleep and disappears in REM sleep. This results in an increase in the upper airway resistance in REM sleep[17].

The decreased sensitivity of the respiratory neurons to carbon dioxide, inhibition of the reticular activating system (RAS), and alteration of metabolic control of respiratory neurons during sleep result in a decrement of tidal volume, minute ventilation, and alveolar ventilation. Hypercapnic as well as hypoxic ventilatory responses decrease during REM and NREM sleep. There is a marked decrease during REM sleep. There are several factors that contribute to the decrements, such as fewer functional medullary respiratory neurons during sleep, decreased sensitivity in the central chemoreceptors and increased resistance in the upper airway. The arousal responses also decrease especially during REM sleep. The voluntary control system may be active during some portion of REM sleep. Therefore, respiration is vulnerable during sleep in normal individuals[17].

Table 2. Physiological changes during wakefulness, NREM Sleep, and REM Sleep[17].

Physiology	Wakefulness	NREM sleep	REM sleep
Parasympathetic activity	++	+++	++++
Sympathetic activity or variable (++)	++	+	Decreases
Heart rate	Normal sinus rhythm	Bradycardia	Brady/tachyarrhythmia
Blood pressure	Normal	Decreases	Variable
Cardiac output	Normal	Decreases	Decreases further
Peripheral vascular resistance	Normal	Normal or decreases slightly	Decreases further
Respiratory rate	Normal	Decreases	Variable; apneas may occur
Alveolar ventilation	Normal	Decreases	Decreases further
Upper airway muscle tone	++	+	Decreases or absent
Upper airway resistance	++	+++	++++
Hypoxic & hypercapnic ventilatory responses	Normal	Decreases	Decreases further
Cerebral blood flow	++	+	+++
Thermoregulation	++	+	–
Gastric acid secretion	Normal	Variable	Variable
Gastric motility	Normal	Decreases	Decreases
Swallowing	Normal	Decreases	Decreases
Salivary flow	Normal	Decreases	Decreases
Migrating motor complex (a special type of intestinal motor activity)	Normal	Slow velocity	Slow velocity
Penile or clitoral tumescence	Normal	Normal	Increases markedly

+, Mild; ++, moderate; +++, marked; ++++, very marked; –, absent; *NREM*, nonrapid eye movement; *REM*, rapid eye movement.

Sleep-wake regulation

The sleep-wake system is regulated by the interaction of two major processes; the one that promotes sleep (process S) and the one that maintains wakefulness (process C)[16].

The need for sleep (process S) accumulates throughout the day and peaks just before sleeping at night and disappears throughout the night. Process C promotes wakefulness and is regulated by the circadian system. Process C accumulates during the day and counteracts process S and promotes wakefulness and alertness[16].

In 1949, Moruzzi and Magoun[16] performed studies dealing with sleep and wakefulness. They concluded that transitions from sleep to wakefulness or from the less extreme states of relaxation and drowsiness to alertness and attention are all characterized by an apparent breaking up of the synchronization of discharge of the

elements of the cerebral cortex[16]. A change marked in the EEG by the replacement of high voltage, slow waves with low-voltage fast activity[16].

An earlier theory of sleep was that the excitatory areas of the upper brain stem, the RAS, simply became fatigued during the waking day and became inactive as a result[25]. An important experiment changed this thinking to the current view that sleep is caused by an active inhibitory process, because it was discovered that transecting the brain stem at the level of the midpons creates a brain cortex that never goes to sleep[25]. In other words, a center located below the midpontile level of the brain stem appears to be required to cause sleep by inhibiting other parts of the brain[25].

Cholinergic neurons appear to be active during waking and REM sleep but not during slow wave sleep, whereas noradrenergic neurons appear more active during waking and slow-wave sleep but inactive during REM sleep. That is, these cell groups appear co-active during certain sleep-wake states, but are communally active during other sleep-wake states[26].

High-frequency electrical stimulation with electrodes implanted in the brainstem reticular formation produced EEG activation and behavioural arousal. These findings seemed to indicate that EEG activation, wakefulness, and consciousness were at one end of a continuum, and EEG synchronization, sleep, and lack of consciousness were at the other end[18].

Neuroanatomical Substrates of Wakefulness

Wakefulness is controlled by the ARAS containing glutamatergic, cholinergic, aminergic and hypocretinergic neurons (Figure 12). Projections from the ARAS terminating in the thalamus and thalamocortical projections to widespread areas of the cerebral cortex produce cerebral cortical activation during wakefulness. Extra thalamic projections from the brainstem reticular neurons terminate in the posterior hypothalamus and the basal forebrain regions. The latter project to the cerebral cortex (basocortical projections) to maintain wakefulness[17].

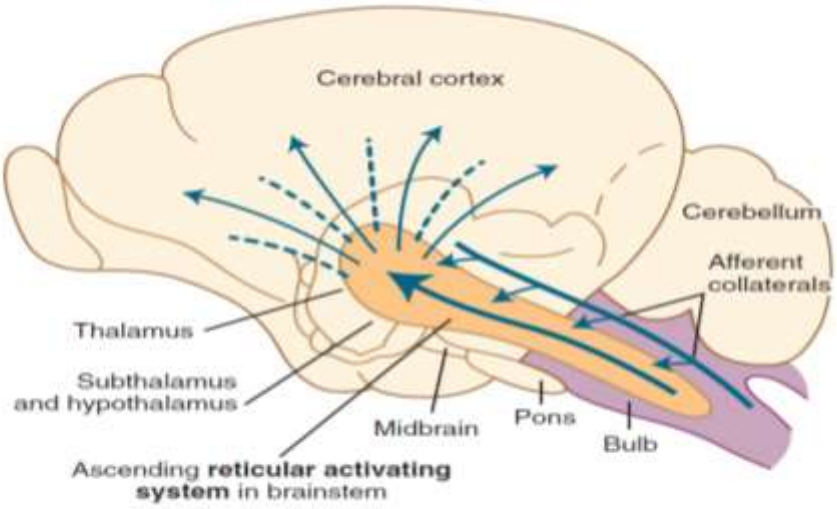


Figure 12: Ascending reticular activating system (ARAS); projections from the ARAS terminating in the thalamus, and thalamocortical projections to widespread areas of the cerebral cortex, inducing cerebral cortical activation during wakefulness[17].

The cholinergic, noradrenergic, dopaminergic and histaminergic neurons are systems regulating the pathways in wakefulness. During wakefulness and during REM sleep the cholinergic neurons fire at the highest rate, but decrease their rates of firing at the onset of NREM sleep. Wakefulness-promoting aminergic neurons include noradrenergic neurons in the locus coeruleus, serotonergic neurons in the dorsal raphe of the brainstem, histaminergic neurons in the tuberomammillary nucleus of the hypothalamus, and possibly also dopaminergic neurons in the ventral tegmental area, substantia nigra, and ventral periaqueductal area[17].

The midbrain dopaminergic system, particularly the dopaminergic neurons play an active role in maintaining wakefulness through their widespread reciprocal connections with sleep/wake regulatory systems of neurons. Norepinephrine-containing locus coeruleus neurons show their highest firing rates during wakefulness, their lowest during REM sleep, and intermediate rates during NREM sleep. Pharmacological studies suggest that posterior hypothalamic histaminergic neurons also help maintain wakefulness[17].

The excitatory amino acids, glutamate and aspartate, are intermingled within the ARAS and are present in many neurons projecting to the cerebral cortex, forebrain, and brainstem. These excitatory amino acids are maximally released during wakefulness. Recent discovery of hypothalamic hypocretin neurons and their widespread CNS projections has directed attention to the role of the hypocretin system in sleep/wake regulation[17].

Two neuropeptides in the lateral hypothalamus and perifornical region were described in 1998 that were termed *hypocretin 1* and *hypocretin 2*[17]. Independently in the same year, Sakurai and colleagues[17] (1998) described two neuropeptides in the same region, which they named *orexin A* and *orexin B* (corresponding to hypocretin 1 and hypocretin 2, respectively). It was shown thereafter that these hypocretin systems have widespread ascending and descending projections to the locus coeruleus, dorsal raphe, ventral tegmental area, tuberomammillary nuclei of the posterior hypothalamus, laterodorsal tegmental (LDT), pedunculopontine tegmental (PPT) nuclei and ventrolateral preoptic (VLPO) neurons in the hypothalamus, basal forebrain, limbic system (hippocampus and amygdala), cerebral cortex, thalamus (intralaminar and midline nuclei), and autonomic neurons (nucleus tractus solitarius [NTS], dorsal vagal nuclei, and intermediolateral neurons of the spinal cord[17]). Hypocretin systems promote wakefulness mainly through excitation of tuberomammillary histaminergic, locus coeruleus noradrenergic, and midline raphe serotonergic neurons as well as dopaminergic neurons. Reduced activity of hypocretin systems may be partly responsible for inducing sleepiness. These systems also suppress REM sleep through activation of the aminergic neurons (REM-off), which in turn inhibit REM-on neurons in the LDT/PPT nuclei. Brainstem arousal centers were identified and characterized, and support was later provided for the concept of *sleep-promoting circuitry* in the anterior hypothalamus/preoptic area[17]. It was later in the mid-1990s that the identity of this sleep-promoting circuitry was revealed[17]. In these investigations, it was demonstrated that the VLPO nucleus contains sleep-active cells that contain the inhibitory neurotransmitters, γ-aminobutyric acid (GABA) and galanin (Gal)[17].

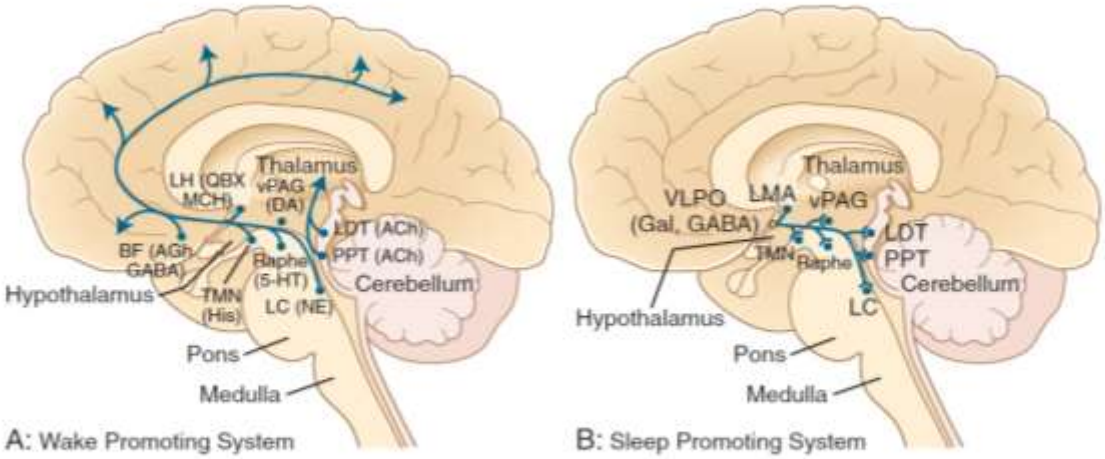


Figure 13: Neurochemistry of brainstem arousal centres[17].

As seen in figure 13 A, the ARAS consists of noradrenergic neurons containing norepinephrine (NE) in the vicinity of the ventrolateral medulla and locus coeruleus (LC), cholinergic neurons (ACh) in the PPT/LDT nuclei, 5-HT (5-Hydroxytryptamine) serotoninergic neurons in the dorsal raphe nucleus (Raphe), dopaminergic neurons (DA) of the ventral periaqueductal gray matter (vPAG), and histaminergic neurons (His) of the tuberomammillary nucleus (TMN). Cortical arousal is generated through two systems: a dorsal route through the thalamus and a ventral route through the hypothalamus and basal forebrain (BF). The latter pathway receives contributions from the orexin (ORX) and melanin-concentrating hormone (MCH) neurons of the lateral hypothalamic (LH) area as well as from GABA-ergic or cholinergic neurons of the BF. These ascending pathways traverse the region at the midbrain-diencephalic junction, lesions in this region causes hypersomnolence. In picture B figure 13, the depiction of sleep-promoting circuitry in the anterior hypothalamus/preoptic area are explained. The VLPO nucleus contains sleep-active cells that contain the inhibitory neurotransmitters, GABA and GAL. The VLPO *(open circle)* projects to all the main components of the ascending arousal system. Inhibition of the arousal system by the VLPO during sleep is critical for maintenance and consolidation of sleep[17].

Change in Sleep Architecture with Age

Sleep patterns and requirements change considerably from infancy to old age. Changes include overall sleep efficiency, the percentage time spent in each sleep stage, how sleep is initiated and maintained. A general trend is that sleep efficiency is reduced with age[16-17] (Figure 14).

A newborn infant spends about 50% of the time in REM sleep, at age 6 years this time is decreased to the normal adult pattern of 25 %[17]. Sleep architecture continues to change with age throughout adulthood. As an individual ages (20 to 60 years of age), SWS declines at a rate of about 2% per decade[16].

Problematic sleep has an adverse effect on all individuals, regardless of their age. Older people generally show an increase in disturbed sleep that can create a negative impact on their quality of life, mood and cognitive abilities. Although the ability to sleep becomes more difficult in the elderly population, the need to sleep does not decrease with age. Difficulty in initiating and maintaining sleep is cited in 43% of the elderly[16,27]. Older people also experience a decrease in melatonin, which may be due to the deterioration of the hypothalamic nuclei which are responsible for the circadian rhythms[28].

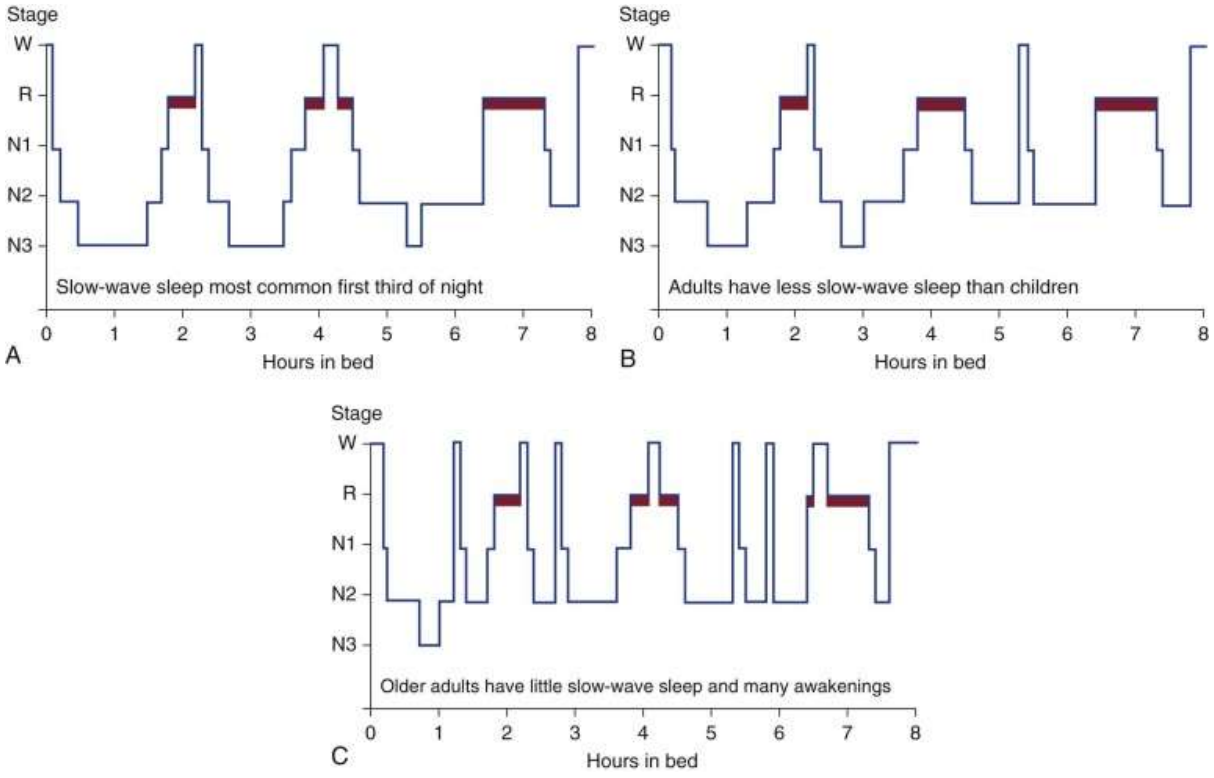


Figure 14: Illustrative histograms showing the changes in sleep with aging.
(A) Shown are a child, (B) a young to middle-aged adult, and (C) an older adult. SWS is maximal in children and decreases with aging. It is minimal or absent in the elderly. The same holds true for REM sleep, which diminishes to a lesser extent and becomes more fragmented with aging. Older adults also have prolonged sleep latency compared with younger subjects; in addition, arousals are more frequent, and sleep efficiency is reduced ([total sleep time/total recording time] × 100) [19].

Function of Sleep

The functions of sleep are poorly understood and sleep remains the greatest biological mystery of all times[17-18]. Sleep deprivation studies in humans have demonstrated impaired performance, which demonstrates the need for sleep. Sleep deprivation studies have shown that sleep deprivation causes sleepiness and poor performance, vigilance, attention, concentration and memory. Sleep deprivation have also been shown to cause some metabolic, hormonal and immunological effects[16].

It has been suggested that sleep might be involved in energy conservation by reducing the core temperature and lowering the metabolic rate by 10% when compared with quiet wakefulness[18]. Sleep may also have benefits in restoring body and brain tissue, memory reinforcement and consolidations[18].

The public health consequence of sleep loss and sleep-related disorders can be devastating. The most visible consequences are errors in judgement contributing to disastrous events. Less visible consequences of sleep disorders are far more prevalent and have far reaching effects on a number of public health indicators: mortality, morbidity, functioning and quality of life, family well-being and health care utilization[16].

Obstructive Sleep Apnea

OSA is a common sleep disorder that affects 2% to 7% of the general population[14] and according to Aseffa *et al*. 2016 sleep apnea affects 5-15% of the general population[29]. The prevalence in men is 4% and 2% in women among a middle-aged population. OSA is a serious medical disorder that has many effects on a patient's health and quality of life and their partners. The prevalence increases with age and is estimated at around 28%-67% for elderly men and 20%-50% for elderly women[30]. OSA is more common in obese and older individuals and weight gain is associated with worsening of OSA[31].

However, only 0.5% of patients with symptoms suggestive of OSA syndrome are evaluated and treated for the disorder[32]. The failure to recognise OSA is in part due to lack of training in sleep medicine and a general lack of awareness. The first step is to increase awareness among doctors and for a simple sleep history to become part of the normal systems review taught at medical school (Table 3)[33].

Table 3. How OSA might present to non-sleep specialist[33].

Cardiologist	Hypertension Left ventricular hypertrophy Nocturnal angina Myocardial infarction Arrhythmias, particularly bradyarrhythmia's Heart failure Cor pulmonale Increased pulmonary artery pressure
Psychiatrist	Depression Anxiety Behavioural problems Acute delirium
Neurologist	Refractory epilepsy Stroke Impaired rehabilitation post stroke Headache on waking
Anaesthetist	Difficult intubation Sensitivity to opioid analgesia and sedation Witnessed apnea's during recovery
Urologist	Nocturia Impotence Erectile dysfunction
Endocrinologist	Hypothyroidism Acromegaly Diabetes
ENT Surgeon	Snoring Sore throat Hoarse voice
Gastroenterologist	Oesophageal reflux
Haematologist	Polycythaemia
Respiratory physician	Nocturnal shortness of breath Respiratory failure

OSA is characterised by upper airway obstruction during sleep[34]. It is a sleep disorder in which the upper airway partially or completely collapses during sleep. Blocking of the airflow results in arousals and awakenings[31]. The term apnea is used if complete obstruction occurred and hypopnea if the obstruction is partial. The obstructions results in repetitive breathing pauses accompanied by low oxygen saturation and arousal from sleep; results in excessive day time sleepiness and can lead to cognitive impairment and increased cardiovascular morbidity. The term obstructive apnea is defined as a cessation of airflow with continued effort for at least 10 seconds[34] (Figure 15).

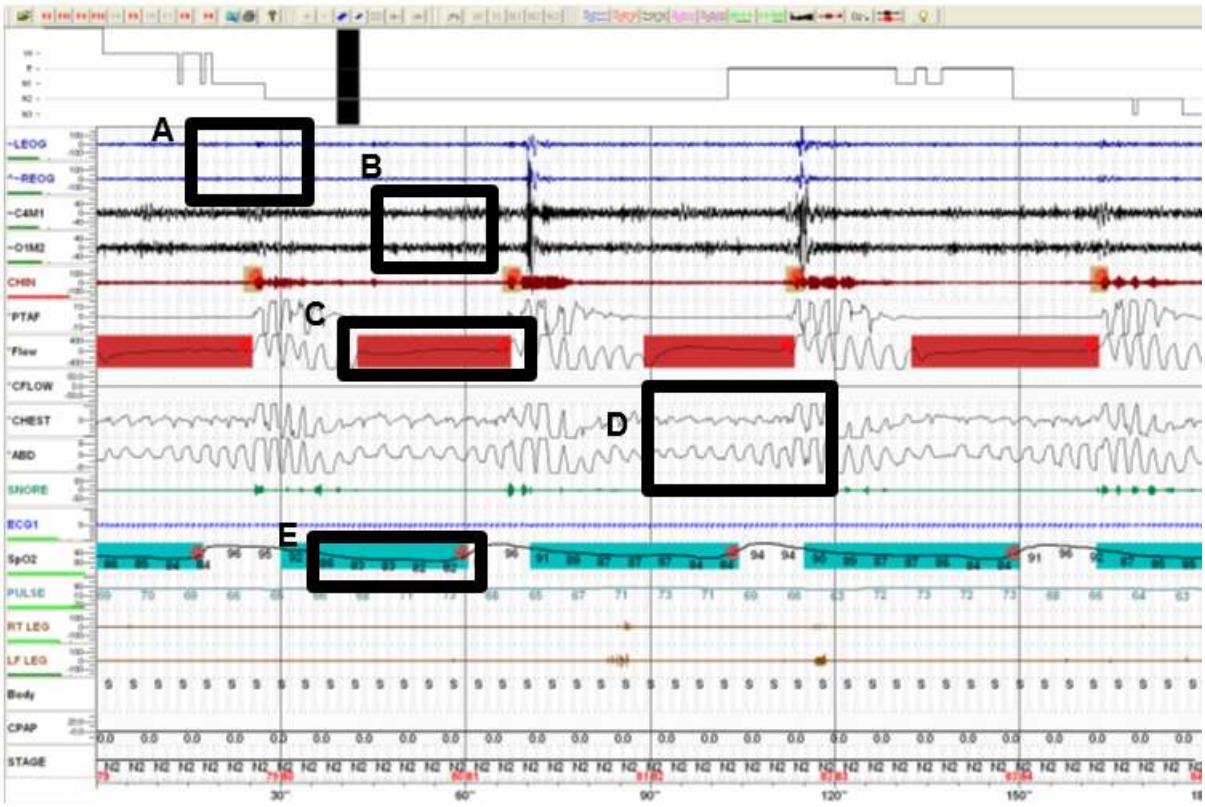


Figure 15: An example of a PSG study with common OSA indicators. A – EOG channels for eye movements; B – EEG channels to determine sleep stages; C – Apnea period; D – Decrease in thoracic and abdomen effort; E – Desaturations. Adapted by Liechka Groenewald.

Patients with a smaller airway opening and changes in the muscle surrounding the airway that make it more collapsible than normal are prone to OSA. People who are obese and or have a large neck circumference are at an increased risk of airway obstruction or closure because of the excessive tissue which may narrow the airway

opening. During wakefulness OSA patients are able to keep their airway open but when asleep, the airway muscles relax thus increasing their risk of airway closure. During airway closure the patient is stimulated to take a deep breath resulting in gasping, choking, snorting and ultimately awakening. After a few breaths the patient returns to sleep and this cycle starts again. OSA should be suspected in anyone who is excessively sleepy during the day and is known to snore, gasp or choke while sleeping[14].

The most common complaint during wakefulness is excessive daytime sleepiness (EDS). However, EDS is not present in all patients with OSA. Nocturnal symptoms are sometimes apparent to the patient, but are generally reported by a bed partner. The most common symptoms during sleep are snoring, snorting, choking attacks and witnessed apnea's. An absence of snoring does not exclude the diagnosis of OSA[33].

OSA is a risk factor for stroke and death from any cause, independent of certain other factors including hypertension, hyperlipidaemia, smoking, body mass index (BMI), diabetes mellitus, atrial fibrillation, gender, race and age[31].

Clinical features of OSA include obesity (BMI >30kg/m^2), a large neck circumference (>40cm), narrow mandible and maxilla, enlarged tonsils and adenoids. However, patient history and clinical examination alone can predict the presence of OSA in only 50% of patients attending a sleep clinic[33].

Cushing's syndrome

Introduction

Cushing's syndrome results from chronic exposure to excess glucocorticoids, which can be a form of either exogenous pharmacological doses of corticosteroids or from an endogenous source of cortisol[35]. All causes of inappropriately high cortisol secretion are grouped in the term "Cushing's syndrome". Irrespective of the underlying etiology, glucocorticoid excess is characterized by a disruption of cortisol circadian rhythmicity and altered feedback mechanisms[36].

Cushing's syndrome patients have an increased mortality rate primarily due to the cardiovascular events induced by glucocorticoid excess related severe metabolic changes[37]. Glucose metabolism abnormalities are common in Cushing's syndrome due to increased gluconeogenesis. This causes disruption of the insulin signaling with reduced glucose uptake and disposal of glucose and altered insulin secretion. This will have an effect on the liver, muscle, adipose tissue as well as the pancreas. Dyslipidemia is a frequent feature due to increased lipolysis, lipid mobilization, liponeogenesis and adipogenesis. Protein metabolism is also affected which can lead to muscle loss. Patients see changes in body composition with fat redistribution resulting in accumulation of central adipose tissue. Metabolic changes, altered adipokine release, glucocorticoid induced heart and vascular abnormalities, hypertension and atherosclerosis contribute to the increased cardiovascular morbidity and mortality[37] (Figure 16).

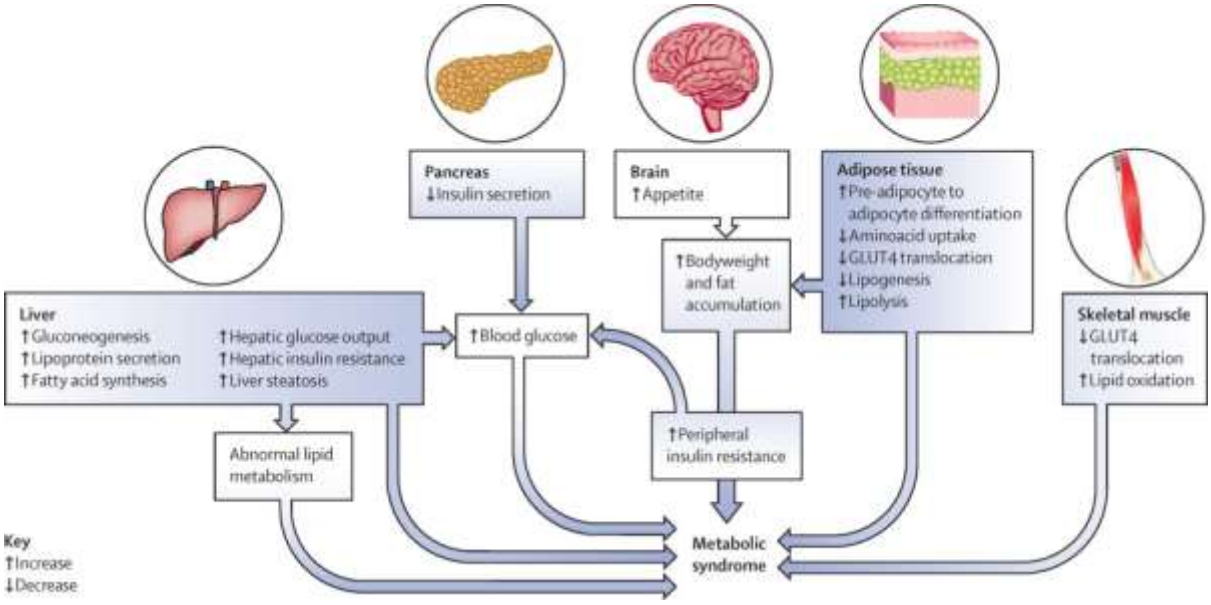


Figure 16: Main pathogenic mechanisms underlying the development of metabolic syndrome in patients with Cushing's syndrome. Circled images represent the main organs that have a role in the metabolic abnormalities seen in patients with Cushing's syndrome; the text below each organ describes the main mechanisms involved in the pathogenesis of these metabolic abnormalities and the main metabolic abnormalities determining metabolic syndrome in patients with Cushing's syndrome. ↑ indicates increased; ↓ indicates decreased. GLUT4=glucose transporter type 4[38].

Epidemiology and causes

Cushing's syndrome is more common in young women than in men[39] with a female to male ratio of 3:1[40]. The estimated incidence of Cushing's syndrome is quoted as 1/250 000 according to Daniel and Newell-Price, 2017 and 0.2-5.0 per million people per year are diagnosed globally[35]. The median age of onset diagnosis is 41.4 years[40]. According to Lacroix *et al.* there is an increased but variable prevalence of Cushing's syndrome in patients with uncontrolled type 2 diabetes, hypertension or early onset osteoporosis[40].

Endogenous Cushing's syndrome results from prolonged and excessive inappropriate concentrations of circulating cortisol[39]. It is a rare disease with significant mortality and morbidity[36] and the identification of its cause and the achievement of optimum treatment is still challenging[40-41].

Endogenous Cushing's syndrome can be divided into adrenocorticotrophic hormone (ACTH)-dependent causes which accounts for 80% of the cases, and ACTH-independent causes which accounts for 20% of the cases[40,42]. Of the ACTH-dependent cases, 80% is caused by a pituitary adenoma (termed Cushing's disease) and the remaining 20% is caused by ectopic ACTH secretion[39]. The ACTH-secreting pituitary adenoma is sporadic except for rare cases in patients with familial multiple endocrine neoplasia type 1[36]. The ectopic ACTH secretion is mainly as a consequence of neuroendocrine tumours[39], such as medullary thyroid carcinoma and pheochromocytomas which produce CRH, leading to the secretion of excess pituitary ACTH[40]. ACTH-independent Cushing's syndrome is caused by benign adrenal adenomas in 60% of cases and carcinomas in 40% of cases[39].

The causes of anterior pituitary adenomas and Cushing's disease were unclear until the identification of molecular genetic abnormalities[40].

Clinical symptoms of Cushing's syndrome

There are several clinical features of Cushing's syndrome namely: obesity with supraclavicular and cervical fat deposits[36], moon face, hypertension, 'buffalo hump', thin skin, hirsutism, oligomenorrhoea / amenorrhea, hypokalemia, purple striae, impaired glucose tolerance/ diabetes mellitus, proximal muscle weakness caused by muscle atrophy, psychiatric disturbance, osteoporosis, bruising, and acne[39]. Patients with Cushing's syndrome often have a flushed appearance (plethoric) and suffer from sleep disorders[41].

Screening is recommended for individuals in whom a diagnosis of Cushing's syndrome is most likely and should be considered in patients with unusual features for their age. Screening is also necessary in patients with adrenal adenoma's found incidentally on computed tomography (CT) scans performed for other reasons[39-40].

Often, patients are symptomatic for 1-2 years before a diagnosis is confirmed[39]. Cushing's syndrome may mimic common conditions, such as obesity, poorly controlled diabetes and hypertension, and often coexist in patients with metabolic syndrome[42]. There are certain signs that most reliably help distinguish Cushing's syndrome from the obesity associated metabolic syndrome, these symptoms include

thin skin, easy bruising (ecchymoses), proximal myopathy and osteopenia[39]. For patients with unexplained severe features, such as resistant hypertension and osteoporosis, assessment is justified irrespective of age. Proximal muscle weakness, wide purple striae and diminished growth in children seem more specific to Cushing's syndrome, and are noted in more severe cases[40] (Figure 17).

Hypertension, osteopenia, menstrual irregularities and neuropsychological disturbances such as depression, irritability, sleep disturbances, cognitive defects or even frank psychosis further characterize patients with Cushing's syndrome. Clinical chemistry may reveal glucose intolerance or frank diabetes, hypokalemia and leukocytosis[36]. Patients who are affected by Cushing's syndrome often complain of poor sleep quality, sleep disturbances and daytime sleepiness. Cushing's syndrome patients often struggle with middle-night and early morning awakenings[10].

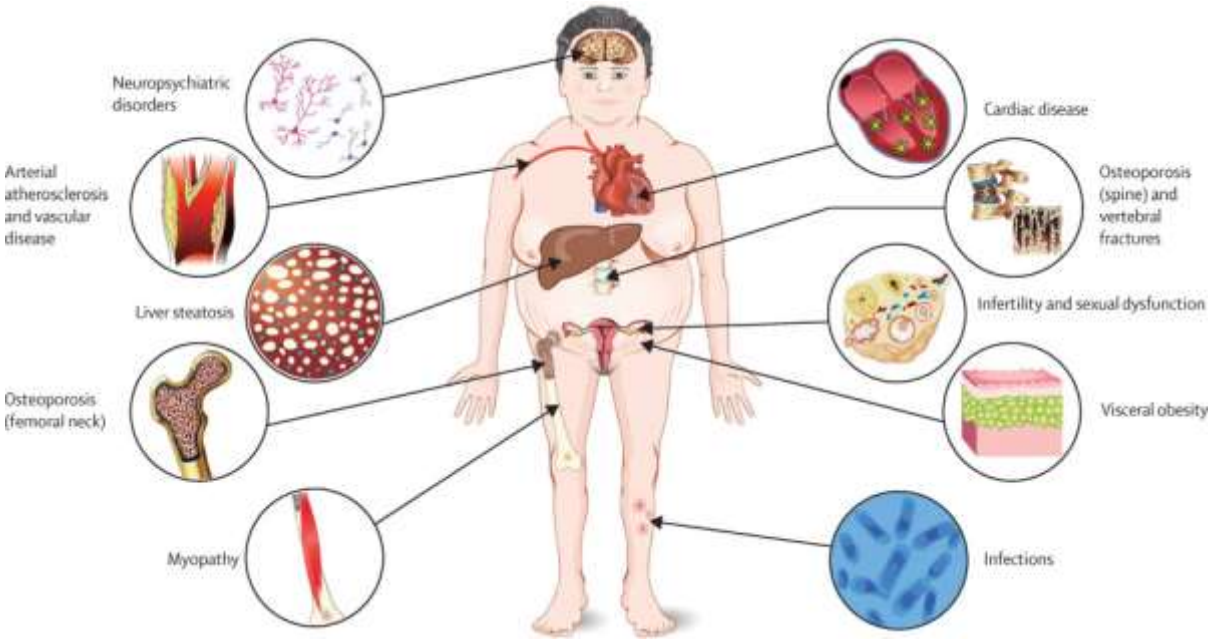


Figure 17: Main comorbidities and clinical complications associated with mortality in patients with Cushing's syndrome[38].

Diagnosis

The diagnosis of Cushing's syndrome remains difficult despite many advances in diagnostic approaches (Figure 17 and Figure 18). The difficulties lie in both the functional demonstration and the localization of hormonal overproduction. Apart from

the problem of differentiating true Cushing's syndrome from pseudo-Cushing's, ACTH-dependent Cushing's syndrome also poses a particular diagnostic challenge. No single test can accurately determine the location of ACTH excess and therefore a combination of non-invasive imaging, stimulation and suppression tests are advised. If non-invasive tests fail to localise the source of ACTH excess an inferior petrosal sinus sampling (IPSS) should follow[43].

In approximately 40% of cases, ACTH-producing pituitary adenomas in pituitary Cushing's disease are not visualised on magnetic resonance imaging (MRI) of the pituitary. Moreover, non-functioning pituitary microadenomas may occur in up to 10% of the general population. Thus, in patients with ectopic ACTH producing tumours an incidental finding of a pituitary microadenoma, which may falsely attribute the source of ACTH production to the pituitary, is not uncommon. Therefore, isolated MRI findings on either the absence or presence of a pituitary microadenoma is of little value without biochemical evidence in support of the MRI findings[43].

Acute intercurrent illness causes hypercorticolemia and false-positive results for the diagnosis of Cushing's syndrome. For unknown reasons some patients with Cushing's syndrome exhibit cyclical secretion of cortisol, which can fluctuate and remit spontaneously, sometimes over many years[39]. This can cause considerable diagnostic difficulty and reinvestigation at intervals and on several occasions may be required. Oral estrogens increase cortisol-binding globulin and therefore lead to falsely elevated serum cortisol concentration[39]. Oral estrogens should be stopped for 6 weeks before investigations[39].

Principal screening tests that are commonly used to establish the diagnosis includes:

1. MRI of the pituitary gland.
2. High-dose dexamethasone suppression test.
3. CRH or desmopressin (DDAVP) stimulation test.

If an adenoma of more than 6mm is demonstrated and the results of the suppression and stimulation test are concordant and in keeping with a pituitary source of ACTH overproduction the diagnosis of Cushing's disease can be made and surgery can be recommended without IPSS[43].

If IPSS is not suggestive of Cushing's disease, imaging to localise ectopic ACTH producing tumors should follow[43]. In suspected ectopic ACTH secretion, a whole body CT scan may reveal a carcinoma[41].

Some patients have physiological hypercortisolism and minimum features of Cushing's syndrome, but no tumour. These patients have been referred to as having pseudo-Cushing's syndrome based on the biochemical results and various disorders should be considered[40]. Alcoholism and severe depression cause patients to look Cushingoid (pseudo-Cushing's), but tests will usually be normal[41].

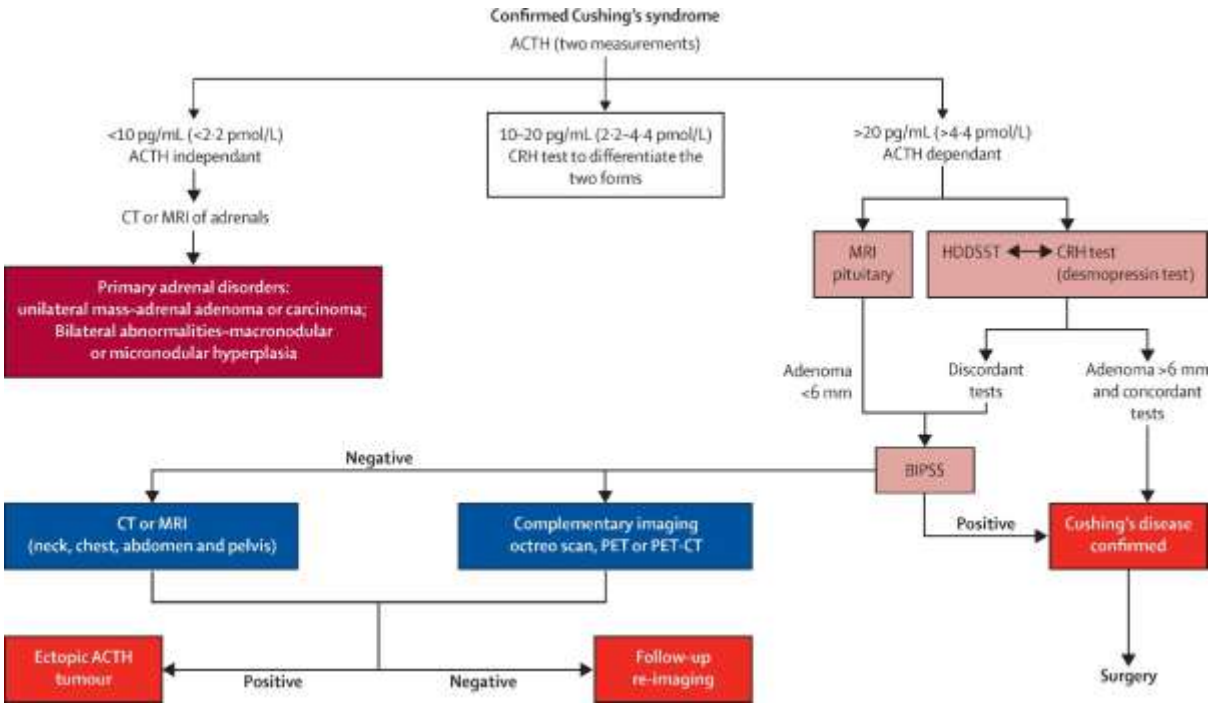


Figure 18: Clinical decision-making flow chart for the differential diagnosis of confirmed Cushing's syndrome of different causes[35].

Complications of Cushing's syndrome

Several complications can arise from Cushing's syndrome such as cardiovascular disease, psychiatric disturbances, endocrine disturbances, ocular complaints, osteoporosis and alterations in calcium metabolism[44] (Figure 19). Patients with osteoporosis and alterations in their calcium metabolism result in pathologic rib and

vertebral fractures in 30-50% of patients with Cushing's syndrome. Additional aseptic necrosis of the femoral or humeral head may occur. Treatment of osteoporosis should be started as soon as possible even in patients with active disease[44].

There is an increase incidence of cardiovascular events in patients with active Cushing's syndrome. Coronary artery disease, congestive heart failure and cardiac infarction are examples. This contributes to a four-fold higher mortality rate compared to the age- and sex-matched population[44]. This increased risk is closely related with features of the metabolic syndrome such as obesity, diabetes, hypertension, hypercoagulability and hyperlipidemia. Hypertension is present in 75-85% of Cushing's diagnosed patients. Abdominal obesity is a known cardiovascular risk factor. Glucose intolerance and frank diabetes affects 20% to 60% of patients with Cushing's syndrome and excess glucocorticoid secretion leads to dyslipidemia[44].

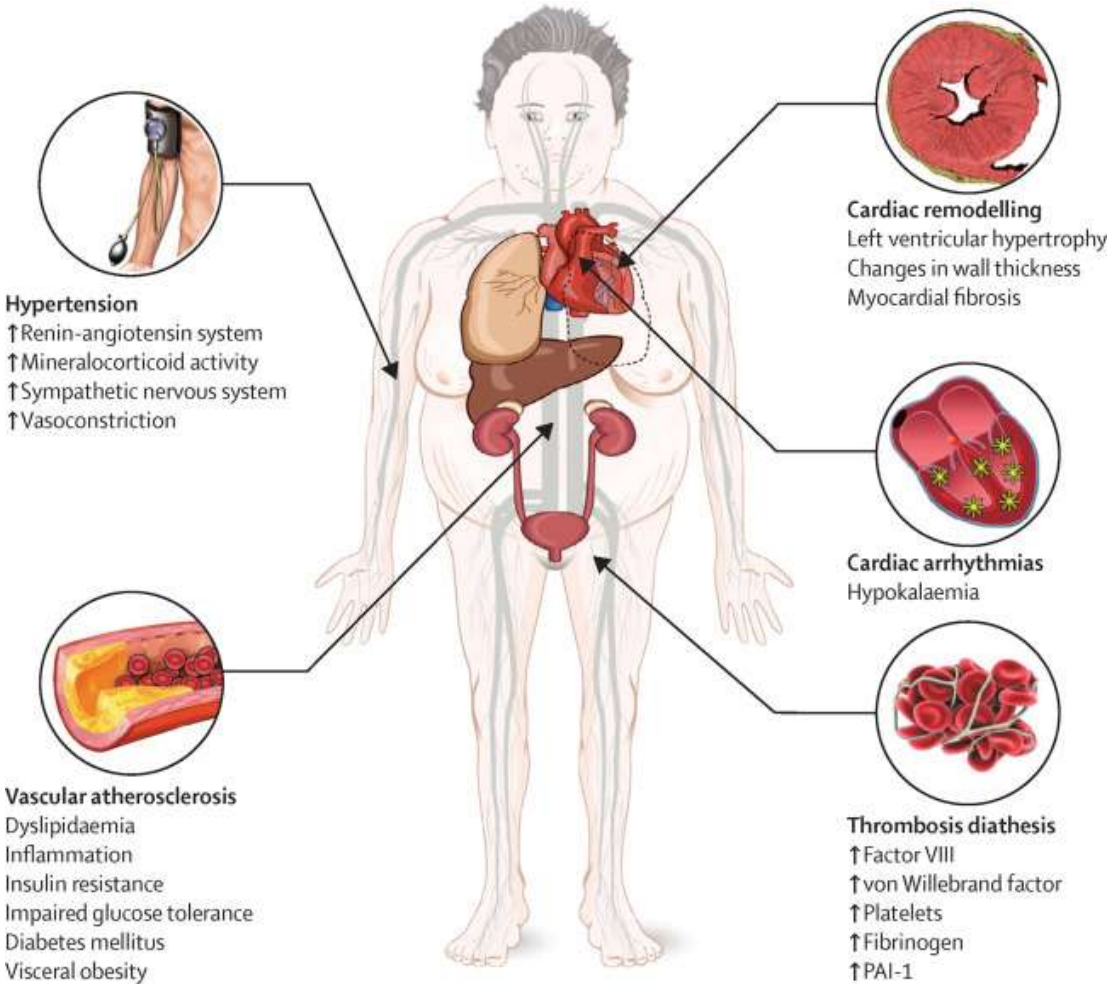


Figure 19: Main pathogenic mechanisms contributing to cardiovascular disease in Cushing's syndrome. ↑ indicates increased; ↓ indicates decreased[44].

Figure 19 shows the tissue abnormalities seen in affected organs, the main pathogenic mechanisms underlying cardiovascular disease in Cushing's syndrome, and the consequent clinical complications. Hypertension, vascular remodelling, and atherosclerosis result from the interplay between several mechanisms regulating plasma volume, peripheral vascular resistance, and cardiac output, all of which are increased in Cushing's syndrome. The mechanisms involved include the renin-angiotensin system, mineralocorticoid activity, the sympathetic nervous system, and the vasoregulatory system. Hypokalaemia increases the risk of malignant ventricular arrhythmias. The pro-inflammatory status, altered angiogenesis, hyperinsulinaemia, and dyslipidaemia all contribute to increased intima-media thickness, development of concentric left-ventricular hypertrophy, impaired diastolic filling, and myocardial fibrosis. A remarkable rise in concentrations of factor VIII, von Willebrand factor, and platelets, and a shortening of the activated partial thromboplastin time, are frequently noted in Cushing's syndrome. PAI-1=plasminogen activator inhibitor type 1[38].

Psychological alterations can occur, ranging from emotional liability, irritability to severe depression. Suicidal behavior and manic episodes affect up to 80% of patients with Cushing's disease. Cognition is often impaired in adult patients[36]. Endocrine changes can also occur. Growth hormone (GH) as well as gonadal function is notably impaired in patients with Cushing's disease. This is a consequence of hypercorticolism or direct pituitary-hypothalamic interactions[36]. Up to a third of patients with Cushing's syndrome has ocular complaints and present with ocular hypertension and exophthalmos[36].

Treatment

Treatments should be initiated as soon as possible in order to avoid progression of the different complications that can arise from Cushing's syndrome. The aim of treatment is to remove the tumour and to normalise ACTH and cortisol secretion while preserving pituitary function[44].

A few measurements can be taken to manage Cushing's syndrome. In most patients with Cushing's disease trans-sphenoidal selective tumour resection (TSS) surgery is

the optimum treatment of choice[36]. The success of TSS depends on the surgeon's expertise because tumours might be small, difficult to recognize, or have dural invasion. After successful tumour resection, concentrations of ACTH and cortisol are low and glucocorticoid replacement is needed[40]. In patients with isolated adrenal adenoma, adrenal surgery is the treatment of choice[39]. Lastly complete excision of the ACTH-secreting tumour results in long lasting remission[39].

Other treatment options include medical therapy to lower the cortisol levels. This can also be used in preparation for surgery in severe cases if surgery is delayed as well as after an unsuccessful surgery[39]. Medical treatment includes steroidogenesis inhibitors, tumour directed drugs and glucocorticoid receptor antagonist, a combination of drugs might be necessary to achieve eucortisolism. Treatment should be individualised considering patient characteristics, drug efficacy and side-effects[40].

Pituitary radiotherapy is another option[36] and is a good primary therapy for non-surgical candidates[40]. Radiotherapy is a second-line approach for persistent or recurrent disease after TSS, particularly when the tumour is invasive and not surgically resectable[40]. ACTH-secreting pituitary tumours are less sensitive to radiation compared to other pituitary tumours[36].

Bilateral adrenalectomy is another treatment for Cushing's syndrome. This method is used when rapid eucortisolism is necessary or when other therapies have failed[36,40]. Candidates for this treatment might include premenopausal woman who desire pregnancy soon after correction of Cushing's syndrome. Laparoscopic adrenalectomy has decreased the morbidity of this procedure. These patients need life-long glucocorticoid and mineralocorticoid replacement and individuals must be educated to avoid acute adrenal insufficiency episodes[40].

Mortality, morbidity and prognosis

The prognosis in patients with Cushing's syndrome that have been inadequately treated has a 5-fold standardized mortality rate[39] post treatment[41-42]. In non-malignant patients with Cushing's syndrome the mortality is increased, with standard mortality ratio (SMR) roughly between 2·0 and 4·0[35]. Cardiovascular deaths are most common. Patients with persisting Cushing's disease after pituitary surgery had a SMR between

4·0 and 5·0[35]. Long term remission improves but does not restore normal SMR. Patients with benign adrenal Cushing's syndrome have normal or increased SMR. Persistent comorbidities such as sleep disorders and hypertension, despite remission, could increase mortality[40].

Chronic hypercortisolism leads to multisystem morbidities. Many cardiovascular risk factors such as obesity, hypertension, diabetes and dyslipidaemia predispose to myocardial infarctions, left ventricular dysfunction and cerebrovascular disease. Cardiovascular risk factors persist in 40-60% of patients and psychopathology and neurocognitive dysfunction will improve to some degree[40].

Chronic brain exposure to excess cortisol causes structural changes in cerebral areas and affects brain functions. Major depression and anxiety disorders are common, but acute psychosis is rare. Cognitive deficits include memory dysfunction, poor visual memory, impaired decision making and sleep disturbances[40]. The immunosuppressive effects of Cushing's syndrome increase susceptibility to sepsis and opportunistic infections (systemic fungal infections)[36]. These comorbidities might not normalise after successful treatment[40].

Timely control of hypercortisolaemia[39], diagnosis and appropriate treatment can alter the course of the disease and are therefore mandatory. Patients cured of Cushing's syndrome require long-term monitoring given the risk of relapse and clinical burden of associated disorders[36,41]. Cardiovascular risks can remain and depression often persists for years after cure[39]. Currently there is a lack of data regarding OSA in Cushing's syndrome.

Sleep apnea in patients with Cushing's syndrome

Sleep apnea has been found to be more common in patients with endocrine disorders such as Cushing's disease and Cushing's syndrome[8]. Patients with Cushing's syndrome may have more sleep disturbances such as insomnia, difficulty in falling asleep or waking during the night or in the early morning[9], fatigue and a multitude of psychiatric syndromes, including frank psychosis and major depression[8]. Most patients complain of daytime fatigue/ sleepiness[9-10]. There is a significant relationship between Cushing's syndrome and other risk factors such as OSA, obesity, diabetes,

dyslipidaemia and hypertension. Cushing's syndrome and OSA has rarely been investigated and was first described in 1992[9]. The above-mentioned symptoms may be worsened by OSA or be caused by steroid induced changes in the sleep architecture. A decrease in delta sleep may explain the insomnia and fatigue and possibly some of the psychiatric symptoms[8].

According to Wang *et al.*[9] patients with Cushing's syndrome present with a significantly higher prevalence of OSA as well a higher apnea/hypopnea index. They found that patients with Cushing's syndrome had a 2.82- fold higher risk of developing OSA[9]. The results supported their hypothesis that patients with Cushing's syndrome regardless of their sex had an increased risk in developing OSA later in life compared with those without Cushing's syndrome[9]. They suggest Cushing's syndrome as an independent risk factor for developing OSA after adjusting for OSA-related physical comorbidities, such as obesity and diabetes mellitus [9].

The definite mechanism underlying the relationship between Cushing's syndrome and OSA risk remains unclear. Cushing's syndrome-related weight gain and centripetal adipose tissue were assumedly to contributing to the development of OSA[9].

A tight bidirectional relationship between the hypothalamus-pituitary-adrenal (HPA) axis and sleep architecture is documented. ACTH and cortisol secretion occur during the first half of the night, during that time SWS prevails. However, ACTH and cortisol peaks are evident during the second half of the evening[10]. REM sleep dominates during the second half of the evenings' sleep cycle[10].

The relationship between hypercortisolism and variations of sleep quality and quantity has not been fully explored. There are only a few studies that have evaluated sleep in Cushing's syndrome using PSG. A full PSG is the gold standard for assessing sleep[10]. It was found that there was reduced SWS, increased in sleep latency, enhanced wake time, a shortened REM latency and elevated REM density[8,10]. There is no South African data regarding OSA in Cushing's syndrome/Cushing's disease.

CHAPTER 3 - RESEARCH DESIGN AND MATERIALS AND METHODS

The purpose of this study was to determine the prevalence of OSA in patients with Cushing's syndrome in a South African setting.

Study Design

Patients who were previously diagnosed with Cushing's syndrome, and were referred to Dr N.P. Ranchhod for a PSG, were included in the study. The PSG was connected and analysed by the primary investigator. Patients who do not have Cushing's syndrome but had an abnormal PSG, indicating OSA, was used to determine the prevalence of sleep apnea. Patients who had a normal PSG were also included to determine the control group's sleep architecture in a hospital setting (Figure 22). The study was done in collaboration with Dr H. Oosthuizen (Endocrinologist), Dr M. Koning (Endocrinologist) and Dr N.P. Ranchhod (Neurologist).

Procedure and equipment

Full details of the PSG procedures were explained to the referred patients. The consent form and sleep questionnaire (Appendix A and B) was discussed and filled in with the patient. The patient sat in a chair. A marker pen and a measuring tape was used to measure where the scalp electrodes were placed. The measurements were done according to the International 10/20 measuring system[45]. The electrodes that were used were the Natus EEG silver cup electrodes. The skin was prepared with Nuprep skin prep gel. The earbud was rubbed against a small area of approximately 1cm by 1cm where the electrode was glued. The preparation of the skin lowers the resistance between the scalp and the electrode to get a clear artefact free recording.

A conductive paste, Ten20 conductive neurodiagnostic electrode paste, was placed between the electrode and the scalp. The electrodes were glued onto the scalp with SLE collodion adhesive. This secures the electrode to stay connected throughout the recording. Electrodes on the face was stuck on with Micropore adhesive tape.

An abdominal band as well as a thoracic band were placed around the patient's chest and stomach area to monitor their breathing movements. The recording device was put around the patient's chest over the thoracic band. The electrodes were connected to the device in the dedicated areas for each electrode. The nasal canula was inserted into the nasal cavity and connected to the device. The pulse oximeter was connected on the patient's finger and connected to the device. The nasal canula was the Embla adult nasal pressure canula with a male luer connector. Micropore adhesive tape was used to secure the electrodes used for eye movements, to secure the pulse oximeter and the nasal canula.

Embla RemLogic PSG from Natus medical (Figure 20), as well as the Trackit™ ambulatory recording system using Polysmith™ software from Nihon Kohden (Figure 21) was used for this project. The equipment monitors the EEG, nasal breathing, thoracic and abdominal breathing movements, leg movements and the blood oxygen levels by means of a pulse oximeter. The recording was done for a minimum duration of 10 hours on each patient. The program used to analyse the recording was the RemLogic™3.4 Read Me software and Polysmith™ software. The RemLogic and Polysmith™ meets the American Academy of Sleep Medicine (AASM) scoring criteria.

The patients were mobile after the equipment was connected. The recording automatically started at 8pm and stopped at 6am the following morning, thus providing data for 10 hours. The sleep period varies from patient to patient; however, all sleep periods are monitored on the EEG channels. The equipment was removed the following morning. The collodion adhesive was removed with acetone and a cotton ball. The recording was downloaded and the analysis were done and interpreted. After the analysis were done a report was generated with the necessary information needed. This includes the percentage of each sleep stage during the evening as well as the apnea-hypopnea index (AHI) of each patient to determine if the patient has sleep apnea or if the study was still within normal limits. All recordings are backed up and stored on external hard drives.

Figure 20: Embla RemLogic apparatus.

Available from: https://stowood.com/product/embla-embletta-and-st-proxy/

Figure 21: Trackit apparatus from Nihon Kohden

Available from: https://www.lifelinesneuro.com/trackit-eeg-ambulatory-amplifier.html

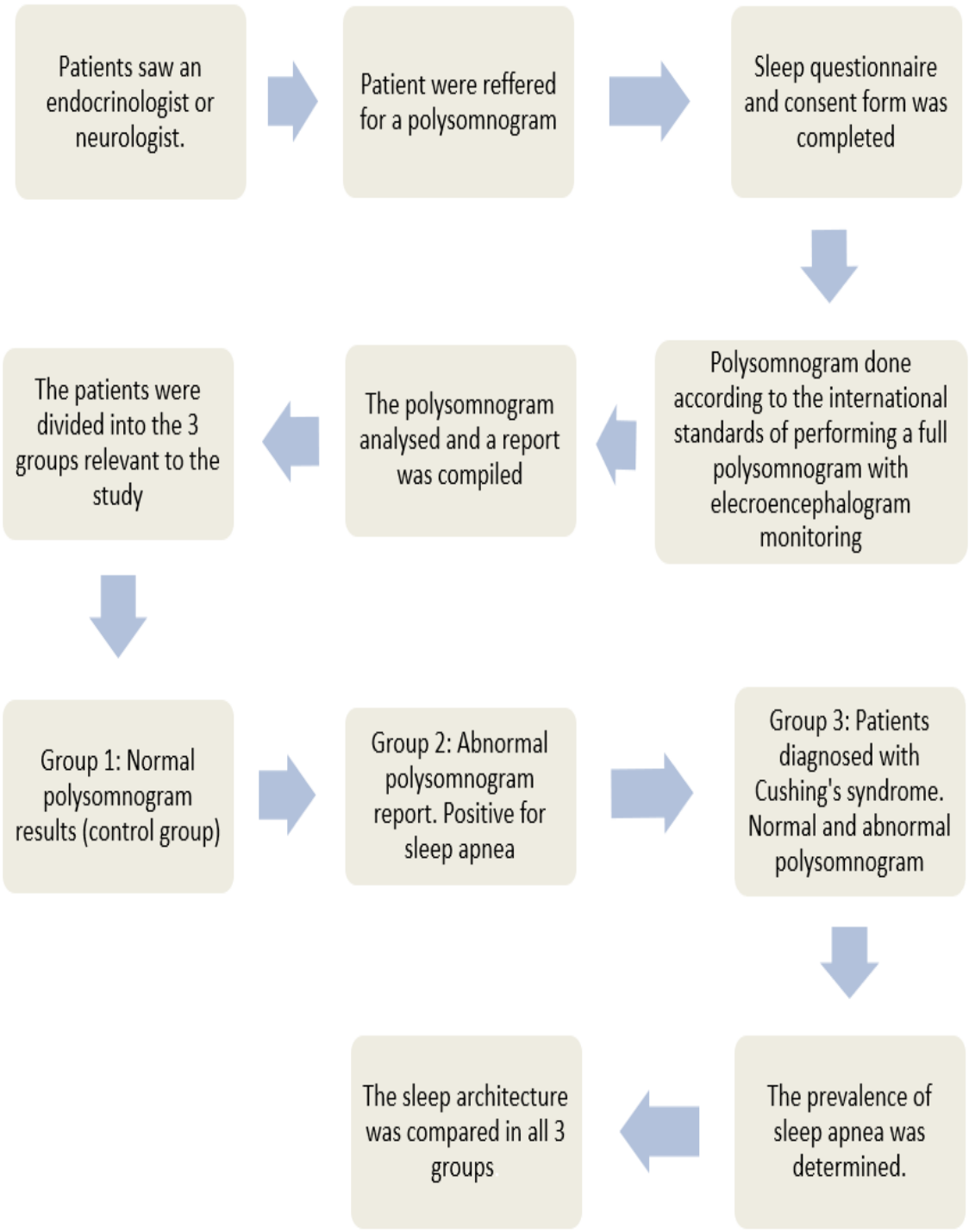


Figure 22: A schematic outline of the study design**.**

Sampling criteria and Procedures

Recruitment:

This was a retrospective as well as a prospective study. Patients that were diagnosed with Cushing's syndrome by Dr H. Oosthuizen and Dr M. Koning and referred for a PSG to the practice of Dr N. P. Ranchhod were included in this study. Patients tested from May 2017 until September 2019 were included in the study. All patient information was handled anonymously. Patients needed to adhere to a selection of inclusion and exclusion criteria to be eligible for this study. Ethics clearance from the University of Pretoria was obtained. Ethics reference number: 131/2019.

The following criteria have been selected to best match the experimental group:

Inclusion criteria for group one, the control group:

- Individuals of any gender.
- Individuals of any race.
- Individuals who are older than 18 years.
- Individuals who presented with OSA signs and symptoms.

Exclusion criteria for group one, the control group:

- Individuals younger than 18 years.
- Patients who were previously diagnosed with OSA.

Inclusion criteria for group two:

- Individuals of any gender.
- Individuals of any race.
- Individuals who are older than 18 years.
- Individuals who presented with any symptoms of sleep apnea.

Exclusion criteria for group two:

- Individuals younger than 18 years.
- Patients who were previously diagnosed with OSA.

Inclusion criteria for group three, the Cushing's syndrome group:

- Individuals of any gender.
- Individuals of any race.
- Individuals who are older than 18 years.
- Individuals diagnosed with Cushing's syndrome.
- Individuals who presented with any symptoms of OSA.

Exclusion criteria for group three, the Cushing's syndrome group:

- Individuals younger than 18 years.
- Patients who were previously diagnosed with OSA.
- Individuals not diagnosed with Cushing's syndrome.

Setting

Referrals from Dr H. Oosthuizen (endocrinologist), Dr M. Koning (endocrinologist) and Dr N.P. Ranchhod (neurologist), for sleep studies were included in this study. The tests were done on patients who were admitted in the hospital. Patients that were diagnosed with Cushing's disease had a PSG done and were included in this study. All the retrospective and prospective patients' medical histories were kept confidential by the treating doctors.

Sample method and sample size

A total number of 222 patients were included in this study. Of the 222 patients only 25 patients were diagnosed with Cushing's syndrome.

Patients that had normal PSGs, as well as abnormal PSGs were included in the study to get an average of the sleep stages in the hospital setting for both normal and abnormal groups separately.

All information obtained during the course of this study was strictly confidential. Data that may be reported in scientific journals will not include any information that identifies any participants in this study.

Statistics

The descriptive statistics, mean, median, standard deviation and inter-quartile range were used to describe the apnea index values and other continuous variables. Frequencies and proportions were used to describe the prevalence of sleep apnea in patients with Cushing's syndrome, as well as other categorical variables. Associations between categorical variables were tested for using the Chi-square test. One-way ANOVA was used to test for a difference in the mean of the percentages for each of the sleep stadia between the normal, abnormal and Cushing's groups. Appropriate post hoc tests were applied. Linear regression was used to compare groups whilst controlling for age as confounder. Pearson's correlation was used to describe the correlation between the percentages in sleep stadia and other continuous variables such as age. Tests were evaluated at 5% level of significance. All analysis was done using STAT 15[46].

Measurements

During the PSG several physiological parameters were measured. To determine the different sleep stages during the evening, an EEG is the best way to monitor the brainwaves. Several scalp electrodes were connected to the patient. These electrodes were connected to specific areas on the scalp according to the international 10/20 system of performing an EEG (Figure 23). During a PSG less electrodes are used to distinguish between the different stages of sleep. Additional EOG, EMG chin electrodes, electro-cardiogram (ECG) and leg electrodes are added for additional information. With this information the different stages of sleep are scored and an overall percentage of each stage of sleep can be determined and compared to the data found in the literature for normal sleep stages.

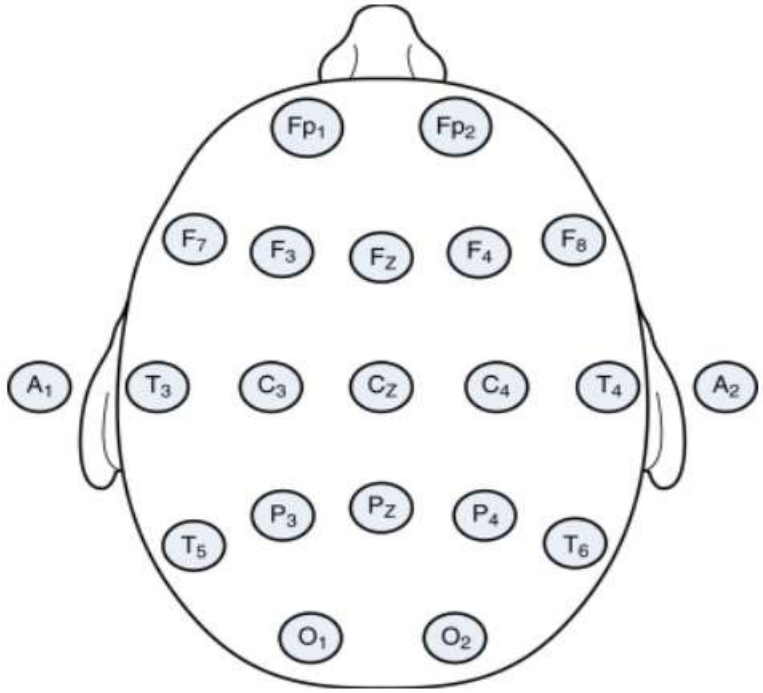


Figure 23: The electrode nomenclature for the original 10-20 electrode system[21].

The patients' breathing was measured with a nasal canula to determine if there were any periods where and apnea or a hypopnea occurred during sleep. These apnea's and hypopneas were measured and an AHI was determined to see if the patients have sleep apnea or a normal PSG. An additional pulse oximeter was added that measures the patient's saturation during the recording (Figure 24). Any changes in the oxygen during the evening were monitored and marked if a desaturation of more than 3% occurs. This determines the desaturation index per hour during the recording. A thoracic as well as an abdominal belt measures the breathing movements on the chest and stomach to determine if the patient has OSA or CSA (Figure 24).

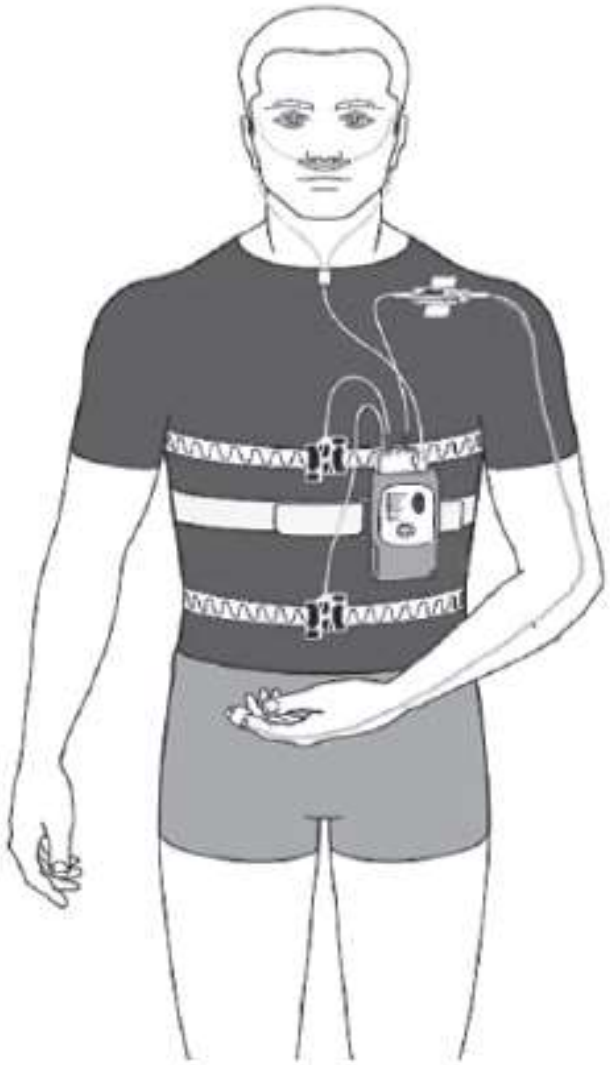


Figure 24: An example of the different equipment used to monitor for sleep apnea[20].

CHAPTER 4 - RESULTS

All Cushing's patients retrospectively and prospectively in the window period May 2017- September 2019 were included in this study to estimate the prevalence of sleep apnea in patients with Cushing's syndrome. A total of 25 Cushing's patients were included with normal and abnormal sleep studies.

In this study a total number of 222 patients were used. A total of 25 (11.26%) patients had Cushing's syndrome, the remaining 197 (88.74%) patients did not have Cushing's syndrome.

Of the 222 patients, 127 (57.21%) were female and 95 (42.79%) were male. Of the 127 females, 108 (85.04%) did not have Cushing's syndrome and 19 (14.96%) had Cushing's syndrome. Of the 95 males, 89 (93.68%) patients did not have Cushing's Syndrome and 6 (6.32) had Cushing's syndrome (Table 4). The Fisher's exact = 0.054 (p-value).

Table 4. The total number of males and females, with and without Cushing's syndrome.

Gender	Total numbers	Non-Cushing's patients	Cushing's patients
Female	127	108	19
Male	95	89	6

Table 5. The total number of female and male patients with normal and abnormal PSGs.

	Normal	Abnormal	Total
Total number of males and females	59 (26.58%)	163 (73.42%)	222 (100%)
Females	48 (81.36%)	79 (48.47%)	
Males	11 (18.64%)	84 (51.53%)	

Table 6. The total numbers of patients who do not have Cushing's syndrome with their total and percentages between normal and abnormal PSGs. The p-value is 0.179.

Non-Cushing's syndrome patients	Total number of patients	Percentage %
Normal	51	25.89
Abnormal	146	74.11
Total	197	100

Table 7. The total number of Cushing syndrome patients with normal and abnormal PSGs. The p-value was 0.179.

Cushing's syndrome patients	Total number of patients	Percentage %
Normal	8	32
Abnormal	17	68
Total	25	100

Of the total amount of patients of 222, 59 patients had normal PSGs and 163 patients had an abnormal PSG. The abnormal PSG were subdivided in mild, moderate and severe sleep apnea (Table 5).

Table 8. The total number and percentages of abnormal PSGs in the total sample group.

Severity	Total of 163 patients	Percentage (%) of the total abnormal patients
Mild	56	34.36
Moderate	42	25.77
Severe	65	39.77

The AHI index was used to compare the severity of sleep apnea between the Cushing's and non-Cushing's syndrome group. The AHI score was divided into 3 groups according to the severity of their sleep apnea. The 3 groups were mild, moderate and severe depending on their polysomnogram results (Table 8 & Table 9).

Table 9. The total number of patients with OSA in Cushing's syndrome and without Cushing's syndrome with their AHI index and severity. The total number of patients as well as the percentages are displayed. P value = 0.519.

AHI index	Non-Cushing's syndrome patients N=197	Cushing's syndrome patients N=25
Normal AHI <5	**51**	**8**
Mild AHI >5-15	**52**	**4**
Moderate AHI >15-30	**35**	**7**
Severe AHI >30	**59**	**6**

Table10. Gender differentiation in patients with abnormal sleep studies with Cushing's syndrome.

Gender	Total (n=17)	Percentage
Male	6	35.3
Female	11	64.7

Table 11. Gender differentiation in patients with normal sleep studies with Cushing's syndrome.

Gender	Total (n=8)	Percentage
Male	0	0
Female	8	100

Table 12. Descriptive statistics: Cross tabulation for continuous variables of non-Cushing' s syndrome patients as well as Cushing' s syndrome patients.

Total with non-Cushing and Cushing' s syndrome patients									
AHI index	Rem sleep%	Stage III sleep%	Stage II sleep%	Stage I sleep%	BMI (kg/m^2)	Height (m)	Weight (kg)	Age (years)	Variable
222	222	222	222	222	209	209	215	222	N
0.21	0	0	0	1.7	19.15	1.45	46	17	Min
104.6	31.5	45.8	90.5	100	72.78	2	179.4	89	Max
23.61	9.58	8.99	60.98	20.51	32.80	1.70	96.05	53.41	Mean
24.83	7.53	7.11	15.24	16.41	8.22	0.11	25.78	15.20	SD
13.78	8.4	7.95	61.95	16	31.14	1.7	90	53	P50
4.45	3.2	3.3	55.2	10.8	27.48	1.62	79	42	P25
36.67	15.4	15.2	70.8	23.6	36	1.79	108	66	P75

Table 13. Descriptive statistics: Cross tabulation for continuous variables of non-Cushing's syndrome patients.

Non-Cushing' s syndrome patients									
AHI index	Rem sleep%	Stage III sleep%	Stage II sleep%	Stage I sleep%	BMI (kg/m^2)	Height (m)	Weight (kg)	Age (years)	Variable
197	197	197	197	197	189	189	194	197	N
0.21	0	0	0	1.7	19.15	1.45	46	18	Min
104.6	31.5	34.6	90.5	100	72.78	2	179.4	89	Max
23.69	9.41	8.59	60.84	21.27	32.37	1.70	94.69	54.39	Mean
24.79	7.48	6.66	15.82	17.16	8.15	0.11	25.03	15.27	SD
13.76	8.3	7.7	62.3	16.4	30.61	1.7	90	55	P50
4.45	3.1	3	55.2	11	27.24	1.62	78	44	P25
36.67	15.3	12.5	70.8	25	35.01	1.79	106	66	P75

Table 14. Descriptive statistics: Cross tabulation for continuous variables of Cushing's syndrome patients.

Cushing' s syndrome patients									
AHI index	Rem sleep%	Stage III sleep%	Stage II sleep%	Stage I sleep%	BMI (kg/m^2)	Height (m)	Weight (kg)	Age (years)	Variable
25	25	25	25	25	20	20	21	25	N
0.3	0	0.8	46.7	7	26.70	1.47	79	17	Min
86.61	25.9	45.8	81.2	27	55.39	1.88	175	69	Max
23.00	10.89	12.10	62.08	14.5	36.91	1.69	108.59	45.68	Mean
25.71	7.91	9.56	9.75	5.68	7.97	0.12	29.68	12.41	SD
15.2	9.1	10.3	59.8	13	34.92	1.72	101	46	P50
4.5	6.6	5.3	56.3	9.9	30.06	1.62	83	37	P25
28.8	18.3	15.2	70.2	18.9	41.73	1.8	132.5	53	P75

Table 15. Descriptive statistics: Cross tabulation for continuous variables of patients with normal PSGs with and without Cushing's syndrome.

Normal studies of non-Cushing' s and Cushing' s syndrome patients									
AHI index	Rem sleep%	Stage III sleep%	Stage II sleep%	Stage I sleep%	BMI (kg/m^2)	Height (m)	Weight (kg)	Age (years)	Variable
71	71	71	71	71	65	65	67	59	N
0.21	0	0	31.2	1.7	20.14	1.45	53.5	18	Min
78.1	27.2	22.3	86.8	45.6	47.34	1.88	150	70	Max
3.55	12.36	8.55	64.56	14.52	30.37	1.68	87.03	43.29	Mean
9.15	7.62	5.33	11.99	8.54	5.41	0.10	20.40	12.74	SD
1.98	12.1	8.4	66	12.9	29.39	1.68	84	43	P50
0.9	5.7	4.6	57.7	8.8	26.96	1.6	74.2	33	P25
4.2	18.3	12.4	73.7	21	33.31	1.74	97	54	P75

Table 16. Descriptive statistics: Cross tabulation for continuous variables of patients with normal PSGs without Cushing's syndrome

Normal studies of non-Cushing' s syndrome patients									
AHI index	Rem sleep%	Stage III sleep%	Stage II sleep%	Stage I sleep%	BMI (kg/m^2)	Height (m)	Weight (kg)	Age (years)	Variable
60	60	60	60	60	56	56	58	51	N
0.21	0	0	31.2	1.7	20.14	1.45	53.5	21	Min
5.52	27.2	22.3	86.8	45.6	47.34	1.88	150	70	Max
2.38	11.96	8.16	65.26	14.64	29.86	1.68	85.71	43.63	Mean
1.67	7.52	5.47	12.48	8.98	5.37	0.10	19.93	12.75	SD
1.78	12.65	8	66.6	13.25	28.99	1.68	84	43.5	P50
0.9	5.4	3.3	58.65	7.25	26.14	1.595	72	33	P25
3.86	17.05	11.7	74.9	21	32.62	1.755	92.4	54	P75

Table 17. Descriptive statistics: Cross tabulation for continuous variables of patients with normal PSGs with Cushing's syndrome

Normal studies of Cushing' s syndrome patients									
AHI index	Rem sleep%	Stage III sleep%	Stage II sleep%	Stage I sleep%	BMI ($kg/m^{2)}$	Height (m)	Weight (kg)	Age (years)	Variable
11	11	11	11	11	9	9	9	8	N
0.3	3	4.7	48.2	8.8	27.74	1.55	79.2	18	Min
78.1	25.9	16.2	74.3	27	43.36	1.86	150	61	Max
9.96	14.67	10.69	60.8	13.84	33.51	1.68	95.56	41.45	Mean
22.70	8.11	4.06	8.35	5.87	4.80	0.10	22.59	13.11	SD
3.5	10.5	10.7	59	11.6	33.75	1.68	84	41	P50
0.36	7.9	7	55.3	9.6	29.45	1.64	81	31	P25
5.99	24.2	14.9	67.3	17.9	34.34	1.73	101	51	P75

Table 18. Descriptive statistics: Cross tabulation for continuous variables of patients with abnormal PSGs with and without Cushing's syndrome.

Abnormal studies of non-Cushing' s and Cushing' s syndrome patients									
AHI index	Rem sleep%	Stage III sleep%	Stage II sleep%	Stage I sleep%	BMI ($kg/m^{2)}$	Height (m)	Weight (kg)	Age (years)	Variable
151	151	151	151	151	144	144	148	163	N
6.2	0	0	0	3	19.15	1.45	46	18	Min
104.6	31.5	45.8	90.5	100	72.78	2	179.4	89	Max
33.05	8.27	9.19	59.29	23.32	33.90	1.72	100.12	58.17	Mean
24.28	7.14	7.81	16.32	18.38	9.02	0.11	26.95	13.91	SD
23.49	7.3	7.7	60.4	17.5	32.16	1.7	94.7	59	P50
13.3	1.9	3.1	54.1	13.3	27.68	1.65	80.35	49	P25
48	13.5	13.2	70.2	26.3	38.51	1.8	111	69	P75

Table 19. Descriptive statistics: Cross tabulation for continuous variables of patients with abnormal PSGs without Cushing's syndrome.

Abnormal studies of non-Cushing' s syndrome patients									
AHI index	Rem sleep%	Stage III sleep%	Stage II sleep%	Stage I sleep%	BMI ($kg/m^{2)}$	Height (m)	Weight (kg)	Age (years)	Variable
137	137	137	137	137	133	133	136	146	N
6.2	0	0	0	3	19.15	1.45	46	18	Min
104.6	31.5	34.6	90.5	100	72.78	2	179.4	89	Max
33.03	8.30	8.78	58.90	24.17	33.42	1.72	98.51	59.10	Mean
24.42	7.22	7.13	16.75	19.02	8.88	0.11	26.05	13.86	SD
23.49	7.2	7.5	60.4	17.9	31.65	1.7	92.8	59	P50
13.1	2.1	3	54	13.6	27.68	1.65	80	50	P25
47.7	13.5	12.7	69	27.5	37.34	1.8	110	69	P75

Table 20. Descriptive statistics: Cross tabulation for continuous variables of patients with abnormal PSGs with Cushing's syndrome.

Abnormal studies of Cushing' s syndrome patients									
AHI index	Rem sleep%	Stage III sleep%	Stage II sleep%	Stage I sleep%	BMI ($kg/m^{2)}$	Height (m)	Weight (kg)	Age (years)	Variable
14	14	14	14	14	11	11	12	17	N
8.51	0	0.8	46.7	7	26.70	1.47	79	33	Min
86.61	19.5	45.8	81.2	24.2	55.39	1.88	175	69	Max
33.26	7.91	13.2	63.09	15.02	39.69	1.71	118.37	49	Mean
23.80	6.58	12.37	10.93	5.69	9.12	0.14	31.42	11.18	SD
24.95	7.7	8.7	60.2	13.75	40	1.72	112.35	49.5	P50
15.49	1.9	4.3	57.3	10.8	30.26	1.6	92.7	39	P25
48	12.7	19.3	70.3	21.2	49.51	1.82	137.2	58	P75

Table 21. Estimates of prevalence/proportions of sleep apnea

Male non-Cushing' s syndrome patients	Female non-Cushing' s syndrome patients	Male Cushing' s syndrome patients	Female Cushing' s syndrome patients	Non-Cushing' s syndrome patients	Cushing's syndrome patients	Variable	
89	108	6	19	197	25	N	
0.85	0.56	0.83	0.47	0.69	0.56	Proportion	
0.04	0.05	0.15	0.11	0.03	0.099	Std. Err.	
0.76	0.47	0.36	0.24	0.63	0.35	95% Confirmation Interval	Binomial Exact
0.92	0.66	0.99	0.71	0.76	0.76		

Chapter 5 – DISCUSSION

OSA is a common sleep disorder that affects 2% to 7% of the general population[14]. Sleep apnea has been found to be more common in patients with endocrine disorders such as Cushing's disease and Cushing's syndrome[8]. Cushing's syndrome is at least three times more prevalent in woman than in men. It can occur at any age, but is more frequent during the fourth and sixth decades of life[38].

In the global adult population the prevalence of OSA is approximately 3%-7% in men and 2%-5% in women[19]. It is said that women are characterized as the forgotten gender in OSA[19]. Women with sleep disorders are not identified as readily as men[47] and it is estimated that more than 90% of women with OSA may not be clinically diagnosed[19]. One reason for this is, at the primary care level, men have more severe apnea than women do[47]. They are also less frequently diagnosed or are misdiagnosed as a result of gender-related differences when it comes to symptom presentation and clinical recognition[19]. Due to the misdiagnosis it often leads to underrepresentation and delays in referrals for PSG evaluation. It was reported that men are three times more likely to have sleep apnea due to the underrepresentation of women in referrals to sleep laboratories[47]. The development of OSA is affected by gender differences, including age, upper airway collapsibility, and hormonal factors (menopause and pregnancy)[19]. It is has been reported that the sex difference in prevalence of sleep disorders decreases in the older population[47].

Obesity is a major risk factor for OSA. Complaints of daytime sleepiness may be present in obese subjects even in the absence of OSA. There may be disturbances in sleep architecture, including lighter and more fragmented sleep compared with nonobese controls. Severely obese patients without OSA may have significantly shorter sleep latencies than lean age-matched controls. Excessive daytime sleepiness has been found in 35% of obese subjects (BMI: 40 ± 6 kg/m^2) without OSA compared with 2.7% in age-matched nonobese controls[4]. It has been proposed that excessive daytime sleepiness and fatigue in obese individuals without OSA could be due to a disruption of sleep homeostasis caused by elevated levels of somnogenic proinflammatory cytokines released[4].

Weight change is a critical factor for the progression of the disease; however, OSA may progress over time even in those with stable weight. Obesity is one of the greatest sleep apnea risk factors and is assessed based on BMI. The prevalence of OSA in obese and severely obese patients is nearly twice that of normal-weight adults. Furthermore, patients with mild OSA who gain 10% of their baseline weight have a six-fold risk increase in OSA progression, and an equivalent weight loss can result in more than 20% improvement in OSA severity[4]. The optimal BMI cut-off point to predict OSA is 30 kg/m^2, while a BMI of between 25 and 29.9 kg/m^2 is considered the BMI range for global cardiovascular risk[4]. In this study Cushing's patients with OSA had an average BMI of 55.39 kg/m^2 compared to a Cushing's patients without OSA, who had an average BMI of 43.35 kg/m^2. The heaviest patient weighed 179.4 kg and the skinniest patient weight 46 kg. The mean weight was 96 kg (SD±25.77). The tallest person was 2 m tall and the shortest patient was 1.45 m tall. The mean height was 1.7 m (SD±0.11). The maximum BMI was 72.78 kg/m^2 and the minimum was 19.14 kg/m^2. The mean BMI was 32.8 kg/m^2 (SD±8.22). In the non-Cushing's group, the lightest patient weighed 46kg and the heaviest was 179.4 kg (Table 13). The mean weight was 94.68 kg (SD±25). It is often assumed that overweight patients has a higher prevalence of sleep apnea[30], however in this study it shows that weight is not always the most reliable indication of a patient who might have sleep apnea.

OSA is more prevalent in males than in females, mild OSA affecting 3-28% of suffers, whilst the moderate form is diagnosed in 1-4% of the affected population[48]. Patients with abnormal PSG can further be divided into the severity of their sleep apnea. An AHI score of 0-<5/h is within normal limits, >5-15/h is mild sleep apnea, >15-30 is moderate sleep apnea and >30/h is severe sleep apnea. Of the total sample size 34.36% had a mild form of sleep apnea; 25.77% had moderately sleep apnea and the remaining 39.87% had severe sleep apnea. The greatest percentage of our study group who had an abnormal PSG had severe sleep apnea.

Our study shows that the prevalence of Cushing's syndrome is higher in females (76%) than in males (24%). It was further evaluated by Gokosmanog *et al*.[15] that OSA among 30 female patients with Cushing's syndrome and age-, sex- and BMI matched controls, found that patients with Cushing's syndrome had a significantly higher prevalence of OSA (50% *vs* 23%). Patients with Cushing's syndrome, regardless of

their sex, had an increased risk of developing OSA in later life compared to patients without Cushing's syndrome[15]. It was found that the risk was approximately 3 times higher in the presence of Cushing's syndrome[9].

Several parameters were tested during the PSG and a sleep questionnaire was completed with the patients. Parameters that were included are the patient's age, weight, height, BMI, stages of sleep (I, II, III & REM sleep) as well as the AHI index. This data may be used for future studies.

Our study did not focus on the prevalence of an abnormal PSG related to the patient's age. However, in the total sample size 57% of the patients were female and 43% were male. The total number of normal PSGs were 26.58% and abnormal PSGs were 73.42%. The females with abnormal PSGs made up a total of 48.47% and males 51.53%. In the group diagnosed with Cushing's syndrome the prevalence was higher in females (76%) than in males (24%). These results are in keeping with the literature that Cushing's syndrome is more common in females than in males[35,39].

The cross tabulation for variables of non-Cushing's syndrome and Cushing's syndrome patients showed that the youngest patient was 18 years old and the oldest patient was 89 years of age. The mean age was 53 years (SD±15.2). The eldest patient in this study was a non-Cushing's patient with the age of 89 years old. The eldest Cushing's patient was 70 years old. In both groups the youngest patients were 18 years of age. Bixler *et al*.[49] reported that the effects of age on the prevalence of sleep apnea in the general population remained unclear, because previous studies only focussed on certain populations. Bixler *et al*.[49] also showed that the prevalence of sleep apnea tends to increase with age[49]. It was also observed that the severity of the apnea's (the AHI index) also decreased with age[49]. Bailes *et al*.[47] had similar results to Bixler et al. and found that sleep apnea and the respiratory disorders related to sleep apnea, are very common in the older population but it is significantly underdiagnosed. Although sleep apnea is more prevalent in the elderly population it was found that the younger population tended to have more severe sleep apnea[49]. The estimates of sleep apnea for middle aged adults is less than 10% and increasing to 20–60% in older adults[47]. Kryger *et al*.[19] found that the prevalence of OSA increases from 1% to 2% in men aged 20 to 44 years to 4.7% in those aged 45 to 64 years and

then decreases to 1.7% in those older than 65 years. The prevalence of sleep related breathing disorders in women increases with age[19]. By the age of 50 years, male predominance decreases to the point where prevalence rates among men and women are similar. A relatively high progesterone/oestrogen ratio and low testosterone levels may help protect women against OSA before menopause[19]. The evidence for this is particularly apparent in the fact that OSA is more than three times as common in postmenopausal compared with premenopausal women. Although limited data suggests that the prevalence and severity of OSA may be lower in women, the consequences of the disease are similar[19]. In this study it was found that males do have a slightly higher prevalence of sleep apnea than females (Table 5).

Of the non- Cushing's syndrome group 26.4% had mild sleep apnea, 17% had moderate sleep apnea and 29.49% had severe sleep apnea. In the Cushing's syndrome group, 16% had mild sleep apnea, 28% had moderate sleep apnea and 24% with severe sleep apnea. This study showed that a high percentage of patients tested for sleep apnea had a moderate-high AHI index. A study done by Shipley *et al*.[50] found that 50% of Cushing's syndrome patient's had mild to moderate sleep apnea[50]

In this study 68% of patients with Cushing's syndrome had OSA. A total of 222 patients' data was collected; 25 patients (11%) were diagnosed with Cushing's syndrome. The remaining 197 patients (89%) did not have Cushing's syndrome. Of the 25 patients that were diagnosed with Cushing's syndrome; 8 were normal; all 8 patients were female. The remaining 17 (68%) patients had OSA. A study done by Shipley *et al*.[50] on patients with Cushing's syndrome, found that 50% had mild to moderate sleep apnea. Another study with a much larger sample size (1612 patient's), from the Taiwan National Health Insurance Research Database, mentioned that the association of Cushing's syndrome with OSA itself has rarely been investigated and that 32% of patients with Cushing's syndrome presented with OSA[9].

There are several limitations to this study which includes limited data on the prevalence of OSA in patients diagnosed with Cushing's syndrome. This study was done for degree purposes and time posed a great limitation on the sample size. The

study was only done at one hospital and that the prevalence of Cushing's syndrome, in the general population, is low and this affected the patient recruitment numbers.

Recommendations for further studies would be to have male versus female comparisons as well as age comparisons. Only patients seeking medical advice and/or help were included in this study. A random controlled group might show different results. Further studies can also divide Cushing's syndrome patients and Cushing's disease patients. In this study they were combined into a single group. Patient's family history and lifestyles can also be investigated in future studies.

Chapter 6 - CONCLUSION

OSA is a societal epidemic with far reaching implications both medically and psychologically, as well as having a major economic burden. OSA affects 2-5% of the Western population whilst the prevalence in other parts of the world is largely underestimated.

This is the first study in a South African setting looking at the prevalence of OSA in Cushing's syndrome patients. In this study, sleep apnea was observed in 17 of the 25 (68%) patients with Cushing's syndrome; 76% of them were females. This number is higher compared to other studies.

The definite mechanism underlying the relationship between Cushing's syndrome and OSA risk remains to be elucidated. Several factors such as anatomical, functional and hormonal may contribute to the pathophysiology. Although the mechanism of OSA in Cushing's syndrome have never been investigated, the role of hypercortisolism in the pathogenesis of OSA has been hypothesised[15]. This study did not show significant differences in sleep EEG parameters between patients with Cushing's syndrome with or without sleep apnea. Data about the effects of hypercortisolism on sleep parameters is limited[10].

Patients with Cushing's syndrome are at an increased risk of death from cardiovascular disease, including myocardial infarction, stroke and thromboembolism. Additional cardiovascular problems such as hypertension and dyslipidaemia, obesity and diabetes and psychiatric problems increase mortality and morbidity rates and reduce the quality of life in these patients. The finding of high frequency of OSA in patients with Cushing's syndrome should be considered a risk factor for increased mortality and morbidity and decreased quality of life.

In conclusion, this study is the first in a South African setting to demonstrate the high prevalence of OSA in patients with Cushing's syndrome. In the South African population the prevalence of sleep apnea in patients with Cushing's syndrome is 68% compared to other studies which found a lower percentage[50]. However, the prevalence

of sleep apnea in the general South African population was found to be 74%. This is higher than the South African Cushing's syndrome group and the general global population. Thus, the occurrence of OSA should always be considered in the general population as well as in patients diagnosed with Cushing's syndrome. It appears that sleep apnea in South Africa is much more prevalent and the exact reason for this need to be further elucidated.

References

1. Hall JE, Guyton AC. Guyton and hall textbook of medical physiology. Twelfth edition. ed. Philadelphia, PA: Saunders Elsevier; 2011.

2. Siegel JM. Sleep viewed as a state of adaptive inactivity. Nat Rev Neurosci. 2009; 10(10):747-53. doi:10.1038/nrn2697

3. Normal sleep. Dent Abstr. 2019; 64(3):203-4. doi:10.1016/j.denabs.2018.11.047

4. Kryger MH, Roth T, Dement WC. Principles and practice of sleep medicine. Sixth ed. Philadelphia, PA: Elsevier; 2017.

5. Drager LF, Togeiro SM, Polotsky VY, Lorenzi-Filho G. Obstructive sleep apnea : A cardiometabolic risk in obesity and the metabolic syndrome. J Am Coll Cardiol. 2013; 62(7):569-76. doi:10.1016/j.jacc.2013.05.045

6. Qureshi A, Ballard RD, Nelson HS. Obstructive sleep apnea. J Allergy Clin Immunol. 2003; 112(4):643-51. doi:10.1016/j.jaci.2003.08.031

7. Redline S, Sotres-Alvarez D, Loredo J, Hall M, Patel SR, Ramos A, et al. Sleep-disordered breathing in hispanic/latino individuals of diverse backgrounds. The hispanic community health study/study of latinos. Am J Respir Crit Care Med. 2014; 189(3):335-44. doi:10.1164/rccm.201309-1735OC

8. Rosenow F, McCarthy V, Caruso A. Sleep apnoea in endocrine diseases. J Sleep Res. 1998; 7(1):3-11.

9. Wang LU, Wang TY, Bai YM, Hsu JW, Huang KL, Su TP, et al. Risk of obstructive sleep apnea among patients with Cushing's syndrome: A nationwide longitudinal study. Sleep Med. 2017; 36:44-7.

10. D'Angelo V, Beccuti G, Berardelli R, Karamouzis I, Zichi C, Giordano R, et al. Cushing's syndrome is associated with sleep alterations detected by wrist actigraphy. Pituitary. 2015; 18(6):893-7.

11. Bronstein MD. Cushing's syndrome : Pathophysiology, diagnosis and treatment. 1st ed. New York: Humana Press; 2011.

12. Jameson JL, DeGroot LJ, De Kretser DM, Giudice L, Grossman A, Melmed S, et al. Endocrinology : Adult & pediatric. 7th edition. ed. Philadelphia, PA: Elsevier/Saunders; 2016.

13. Bista B, Beck N. Cushing syndrome. Indian J Pediatr. 2014; 81(2):158-64. doi:10.1007/s12098-013-1203-8

14. Melamed KH, Goldhaber SZ. Obstructive sleep apnea. Circulation. 2015; 132(6):e114-e6.

15. Gokosmanoglu F, Kan EK, Atmaca H, Guzel A. Increased prevalence of obstructive sleep apnea in patients with Cushing's syndrome compared with weight-and age-matched controls. Eur J Endocrinol. 2017; 176(3):267-72.

16. Colten HR, Altevogt BM, Harding K, Feldman M. Sleep disorders and sleep deprivation: An unmet public health problem. J Am Acad Child Adolesc Psychiatry. 2008; 47(4):473-4. doi:10.1097/01.CHI.0000270812.55636.3b

17. Daroff RB, Bradley WG. Bradley's neurology in clinical practice. 6th ed. Philadelphia, PA: Elsevier/Saunders; 2012.

18. Schupp M, Hanning CD. Physiology of sleep. Continuing Education in Anaesthesia, Critical Care & Pain. 2003; 3(3)

19. Kryger MH, Avidan AY, Berry RB. Atlas of clinical sleep medicine. Second edition. ed. Philadelphia, PA: Elsevier/Saunders; 2014.

20. Berry RB. Fundamentals of sleep medicine. Philadelphia: Elsevier/Saunders; 2012.

21. Libenson MH. Practical approach to electroencephalography. Philadelphia, PA: Elsevier/Saunders; 2010.

22. Niedermeyer E, Lopes da Silva FH. Electroencephalography : Basic principles, clinical applications, and related fields. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005.

23. De Weerth C, Zijl RH, Buitelaar JK. Development of cortisol circadian rhythm in infancy. Early Hum Dev. 2003; 73(1-2):39-52.

24. Pineda MH, Dooley MP. Mcdonald's veterinary endocrinology and reproduction. 5th ed. Ames, Iowa: Iowa State Press; 2003.

25. Hall JE. Guyton and hall textbook of medical physiology. 13th ed. Philadelphia, PA: Elsevier; 2016.

26. Garcia-Rill E. Disorders of the reticular activating system. Med Hypotheses. 1997; 49(5):379-87.

27. Foley DJ, Monjan AA, Brown SL, Simonsick EM, Wallace RB, Blazer DG. Sleep complaints among elderly persons: An epidemiologic study of three communities. Sleep. 1995; 18(6):425-32. doi:10.1093/sleep/18.6.425

28. Phillips B, Ancoli-Israel S. Sleep disorders in the elderly. Sleep Med. 2001; 2(2):99-114. doi:10.1016/S1389-9457(00)00083-6

29. Assefa SZ, Diaz-Abad M, Korotinsky A, Tom SE, Scharf SM. Comparison of a simple obstructive sleep apnea screening device with standard in-laboratory polysomnography. Sleep Breath. 2016; 20(2):537-41.

30. Arnold J, Sunilkumar M, Krishna V, Yoganand SP, Kumar MS, Shanmugapriyan D. Obstructive sleep apnea. J Pharm Bioallied Sci. 2017; 9(Suppl 1):S26-S8.

31. Young D, Collop N. Advances in the treatment of obstructive sleep apnea. Curr Treat Options Neurol. 2014; 16(8):1-14. doi:10.1007/s11940-014-0305-6

32. Jay SB, Patel. SR. Obstructive sleep apnea. Ann Intern Med. 2014; 161(9):1.

33. Schlosshan D, Elliott MW. Sleep • 3: Clinical presentation and diagnosis of the obstructive sleep apnoea hypopnoea syndrome. Thorax. 2004; 59(4):347. doi:10.1136/thx.2003.007179

34. Renata LR. Clinical assessment of the obstructive sleep apnoea/hypopnoea syndrome. Ther Adv Respir Dis. 2010; 4(2):83-91. doi:10.1177/1753465810365080

35. Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. Lancet. 2015; 386(9996):913-27.

36. De Martin M, Pecori Giraldi F, Cavagnini F. Cushing's disease. Pituitary. 2006; 9(4):279-87.

37. Ferraù F, Korbonits M. Metabolic comorbidities in Cushing's syndrome. Eur J Endocrinol. 2015; 173(4):133-57. doi:10.1530/EJE-15-0354

38. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BM, Colao A. Complications of Cushing's syndrome: State of the art. Lancet Diabetes Endocrinol. 2016; 4(7):611-29. doi:10.1016/S2213-8587(16)00086-3

39. Daniel E, Newell-Price J. Cushing's syndrome. Medicine (United Kingdom). 2017; 45(8):475-9.

40. Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. The Lancet. 2015;

41. Rees A, Levy M, Lansdown A. Clinical endocrinology and diabetes at a glance. Chichester, West Sussex: John Wiley & Sons; 2017.

42. Prague JK, May S, Whitelaw BC. Cushing's syndrome. BMJ. 2013; 346(mar27 3):f945.

43. Pretorius E, Ascott-Evans B, Davis R, Doruyter A. Latest diagnostic approaches to determine the cause of ACTH-dependent Cushing's syndrome, in the South African setting. J Endocrinol Metab Diabetes South Afr. 2016; 21(3):47-50. doi:10.1080/16089677.2016.1212965

44. De Martin M, Giraldi F, Cavagnini F. Cushing's disease. Pituitary. 2006; 9(4):279-87.

45. Berry RB, Brooks R, Gamaldo C, Harding SM, Lloyd RM, Quan SF, et al. Aasm scoring manual updates for 2017 (version 2.4). Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine. 2017; 13(5):665-6. doi:10.5664/jcsm.6576

46. StataCorp. Stata statistical software: Release 15. College Station, TX: StataCorp LLC; 2017.

47. Bailes S, Baltzan M, Alapin I, Fichten CS, Libman E. Diagnostic indicators of sleep apnea in older women and men: A prospective study. J Psychosom Res. 2005; 59(6):365-73.

48. Ayo-Yusuf OA, Motloba DP, Sethusa MPS, Solomons YF. Obstructive sleep apnoea: Epidemiology, quality of life, and management - implications for dentists. A review. S Afr Dent J. 2015; 70(5):190-5.

49. Bixler EO, Vgontzas AN, Ten Have T, Tyson K, Kales A. Effects of age on sleep apnea in men: I. Prevalence and severity. Am J Respir Crit Care Med. 1998; 157(1):144-8.

50. Shipley JE, Schteingart DE, Tandon R, Pande AC. Eeg sleep in Cushing's disease and Cushing's syndrome: Comparison with patients with major depressive disorder. Biol Psychiatry. 1992; 32(2):146.

Appendix A: Informed consent

Participant Information Leaflet and Informed Consent Document

Research study title: The prevalence of sleep apnea in patients with Cushing's Syndrome

Principal Investigator: Liechka Groenewald

Supervisor: Prof P. du Toit

Daytime and after-hours telephone number:

Daytime number/s: 0829706040 and 0129987320

After hour number: 0829706040

Date and time of first informed consent discussion:

Date	Month	Year	Time

Dear Prospective Participant

Dear Mr. / Mrs.

……

1) Introduction:

You are invited to volunteer in a research study. I am doing research for a Masters degree purpose at the University of Pretoria. The study is to determine what the prevalence of sleep apnea is in Cushing's Syndrome patients. The information in this document is to help you to decide whether you would like to participate in our study and to inform you of the purpose, aim and procedures of this study. Before you agree to take part in this study it is important to fully understand what the study entails and what is involved. If you have any questions, which are not fully explained in this document, please do not hesitate to ask the researcher, Liechka Groenewald. You should not agree to take part unless you are completely happy about all the procedures involved.

2) What is the nature and purpose of this study?

The aim of this study is to evaluate the number of patients with Cushing's syndrome who have sleep apnea and by doing so we wish to learn more about the prevalence

of sleep apnea in patients with Cushing's syndrome. With this information patients can be treated earlier to help with symptoms caused by sleep apnea, such as tiredness, mood changes, daytime sleepiness, diabetes etc.

3) **Explanation of procedures and what would be expected from participants:**

You will be asked to complete a questionnaire regarding your illness. All the information in this questionnaire will be kept confidential and will only be accessible to the main investigator, Liechka Groenewald and the referring doctors involved in the study. None of the information given to us will be shared with anyone and the patient will remain anonymous.

Following completion of the questionnaire we will explain all the details and prepare you for testing. All the equipment that will be used during the study will be connected as well as removed by Liechka Groenewald. There will be electrodes placed on your head using the international 10-20 measuring system. The recording box will be on your chest where all the wires gets connected to. The electrodes are necessary to monitor your brain waves, which is the electrical activity of the brain. The electrodes need to make contact with your scalp and therefore your head will be cleaned with an ear bud and a scrub designed for electroencephalographic (EEG) monitoring. A conduction paste is used between the electrode and the scalp for conductivity. The electrodes will also be glued to the skin with Collodion glue to ensure the wires stay on for the whole duration of the monitoring during wakefulness and sleep.

The device will be applied to patients who was referred for a polysomnogram. The recording will start automatically at approximately 8 pm and record till 6 am. This is necessary to monitor the sleep patterns. The equipment will be removed in the morning and the brainwaves will be analysed by Liechka Groenewald. The specific times the patient slept in each phase of sleep will be noted and used for the study as well as the apnea and hypopnea index will be used to determine if the patient tested positive for sleep apnea. This will conclude the testing procedures.

4) **Possible risk and discomforts involved**

There are no medical risks associated with the study. The only discomfort involved will be experienced while wearing the device. If you as participant have any concerns please feel free to bring it under attention of the investigator at any time.

5) **What is the duration of the study?**

As described above, the testing will be performed when you as a patient was referred for a polysomnogram by the referring doctor. Patients with Cushing's syndrome will be referred by the treating endocrinologist at Pretoria East Hospital. The device will be kept on from the afternoon until the next morning, and then all the equipment will be removed and analysed.

6) **Possible benefits of this study?**

By participating in this research study, you are contributing to an area where research information is very limited. This study will help to make doctors aware of the prevalence of sleep apnea in patients with Cushing's syndrome. The study results may help us improve treatment that patients might need.

7) Compensation

You will not be paid to take part in the study. The test was requested by your treating doctor for medical reasons and not for the sole purpose of this study.

8) What are your rights as a research participant?

Your participation in this study is entirely voluntary and you have the right to withdraw or stop at any time without stating a reason. Your withdrawal will not affect your access to other medical care. You have the right to information relating to the purpose, procedures and any other ethical considerations of the study. The investigator retains the right to withdraw you from the study if it is considered to be in your best interest or if information supplied by you are inaccurate or false; or in the case where you did not follow the guidelines and regulations of the study. A copy of the results and/or study can be made available to you by the referring doctor, once the test and analysis have been completed.

9) Ethics approval?

This Protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, telephone numbers 012 356 3084 / 012 356 3085 and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2013), which deals with the recommendations guiding doctors in biomedical research involving human/subjects. A copy of the Declaration may be obtained from the investigator should you wish to review it.

10) Information

If I have any questions concerning this study, I should contact:
Dr. N.P. Ranchhod, telephone number: 012 998 7320 or Liechka Groenewald, telephone number 0829706040.

11) Confidentiality:

All information obtained during the course of this study will be regarded as confidential. Each participant that is taking part will be provided with an alphanumeric coded number e.g. 001. This will ensure confidentiality of information so collected. Only the researcher will be able to identify you as participant. The results obtained might be published or presented in such a fashion that patients remain unidentifiable. The hard copies of all your records will kept at the practice of Dr. N.P. Ranchhod at Pretoria East Hospital.

12) Consent to participate in this study

- I confirm that the person requesting my consent to take part in this study has told me about the nature and process, any risk or discomforts, and benefits of the study.
- I have also received, read and understood the above written information about the study.
- I have had adequate time to ask questions and I have no objectives to participate in this study.
- I am aware that the information obtained in the study, including personal details, will be anonymously processed and presented in the reporting of results.
- I understand that I will not be penalized in any way should I wish to discontinue with the study and that withdrawal will not affect my further treatments.
- I am participating willingly.
- I have received a signed copy of this informed consent agreement.

_____ _____

Participant's name (Please print) Date

_____ _____

Participant's signature Date

_____ _____

Researcher's name (Please print) Date

_____ _____

Researcher's signature Date

AFFIRMATION OF INFORMED CONSENT BY AN ILLITERATE PARTICIPANT
(if suitable)

I, the undersigned, ……..…., have read and have explained fully to the participant, named …… , the informed consent document, which describes the nature and purpose of the study in which I have asked the him/her to participate. The explanation I have given has mentioned both the possible risks and benefits of the study. The participant indicated that he/she understands that he/she will be free to withdraw from the study at any time for any reason and without jeopardizing the his/hers standard care.

I hereby certify that the patient has agreed to participate in this study.

_____	_____
Participant's name (Please print)	Date
_____	_____
Participant's signature	Date
_____	_____
Investigator's Name (Please print)	Date
_____	_____
Investigator's Signature	Date
_____	_____
Name of the person who witnessed the informed consent (Please print)	Date
_____	_____
Signature of the Witness	Date

SLEEPSTUDY QUESTIONNAIRE

Name:_____

Date of birth:_____

Height: _____cm Weight: _____kg Blood pressure: _____/_____ Neck circumference: _____cm

1. Do you feel refreshed when you wake up in the morning? _____

2. Does someone complain that you stop breathing during the night? _____

3. Do you snore? _____

4. Do you kick a lot with your legs during sleep? _____

5. Do you suffer from any of the following: -

-High blood pressure_____- Diabetes/ insulin resistance_____

- Heart problems _____- Depression _____

- Irritable _____- Forgetful _____

- Poor concentration_____- Headaches in the mornings_____

6. Any lung problems or any other illnesses? _____

7. Sinus problems?_____Cholesterol_____Smoking_____

8. Clear, vivid dreams as soon as you fall asleep?_____

9. Do you sometimes fall when you experience strong emotions? _____

10. Do you sometimes wake up in the morning, aware of your surroundings but unable to move? _____

11. How long does it take to fall asleep? _____

12. Difficulty to stay asleep / wake up often? _____

13. Are you using any medication/sleeping tablets? _____

14. How easily would you fall asleep in the following situations?

Key: **0**= No chance of dozing; **1**= Slight chance of dozing; **2**= Moderate chance of dozing; **3**=High chance of dozing

14.1	Sit and read	0	1	2	3
14.2	Watching TV	0	1	2	3
14.3	Sitting inactive in a public place	0	1	2	3
14.4	As a passenger in a car for an hour without a break	0	1	2	3
14.5	Lying down to rest in the afternoon when possible	0	1	2	3
14.6	Sitting and chatting to someone	0	1	2	3
14.7	Sitting quietly after lunch	0	1	2	3
14.8	IN A car when stopped at a traffic light for a few minutes	0	1	2	3

Total: _____

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

29 April 2019

Approval Certificate
New Application

Ethics Reference No.: 131/2019
Title: The prevalence of sleep apnea in patients with Cushing's syndrome

Dear Miss L Groenewald

The **New Application** as supported by documents received between 2019-03-25 and 2019-04-24 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 2019-04-24.

Please note the following about your ethics approval:

- Ethics Approval is valid for 1 year and needs to be renewed annually by 2020-04-29.
- Please remember to use your protocol number (131/2019) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Additional Conditions:

- Approval is conditional upon the Research Ethics Committee receiving approval from Pretoria East Private Hospital.

Researcher to note: The REC approves the request for a waiver for the need to obtain individual informed consent, for the retrospective part of this study.

We wish you the best with your research.

Yours sincerely

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag X323
Arcadia 0007, South Africa
Tel +27 (0)12 356 3084
Email deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the
United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

30 July 2020

Acknowledgement Certificate
Research Completed

Ethics Reference No.: 131/2019
Title: The prevalence of sleep apnea in patients with Cushing's syndrome

Dear Miss L Groenewald

The **Research Completed Report** as supported by documents received between 2020-06-09 and 2020-07-29 for your research, was acknowledged by the Faculty of Health Sciences Research Ethics Committee on 2020-07-29 as resolved by its quorate meeting.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of
Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African
Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Announcement and instruction to all researchers from the Faculty of Health Sciences Research Ethics Committee

This is an update following previous instructions from the Research Ethics Committee, accounting for recent announcements by government in relation to COVID-19 on 24 May 2020.

All researchers must minimise the risk of transmission at research sites and in studies involving human participants approved by the Research Ethics Committee. To this end,

1) all non-therapeutic or non-interventional research data gathering involving contact with human participants remain suspended, with the exception of studies involving telephonic or other online/remote methods of data collection;
2) research that is entirely situated in a laboratory is permitted provided that COVID-19 precautionary measures are in place;
3) research that is merely utilising existing records or data is permitted provided that COVID-19 precautionary measures are in place
4) emergency research related to COVID-19 is permitted after ethics approval;
5) everyone should endeavour protecting research participants, personnel and students in reducing the risk of transmission of COVID-19.

For therapeutic and clinical research trials:

1) each research study or study site must maintain a plan to minimise exposure to COVID-19 risk for all parties involved in the study, including but not limited to research participants, researchers and student researchers;
2) Whenever feasible, in-person visits should be substituted with telephonic visits;
3) Principal investigators and study sites should maintain measures to ensure that there is no interruption of required medication/essential treatment and monitoring of adverse events;
4) Researchers and study sites should develop a 'COVID-19' template register in case retrospective contact tracing becomes necessary;
5) New enrolments into clinical trials remain suspended. Potential exceptions to this announcement should be discussed with the chair or a deputy chair of the REC;
6) Serious adverse events at an UP-site should be reported on the PeopleSoft system within 72 hours as usual.

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Netcare Hospital Management (Pty) Limited

Tel: + 27 (0)11 301 0000
Fax: Corporate +27 (0)11 301 0499
76 Maude Street, Corner West Street, Sandton, South Africa
Private Bag X34, Benmore, 2010, South Africa

RESEARCH OPERATIONS COMMITTEE FINAL APPROVAL OF RESEARCH

Approval number: UNIV-2019-0027

Ms L Groenewald

E mail: neelay.ranch@gmail.com

Dear Ms Groenewald

RE: PREVALENCE OF SLEEP APNEA IN PATIENTS WITH CUSHING'S SYNDROME

The above-mentioned research was reviewed by the Research Operations Committee's delegated members and it is with pleasure that we inform you that your application to conduct this research at Netcare Pretoria East Hospital, has been approved, subject to the following:

i) Research may now commence with this FINAL APPROVAL from the Netcare Research Operations Committee.

ii) All information regarding Netcare will be treated as legally privileged and confidential.

iii) Netcare's name will not be mentioned without written consent from the Netcare Research Operations Committee.

iv) All legal requirements regarding patient / participant's rights and confidentiality will be complied with.

v) All data extracted may only be used in an anonymised, aggregated format and for the purposes of this specific study as specified in the proposal. The data may under no circumstances be used for any other purpose whatsoever.

vi) The research will be conducted in compliance with the GUIDELINES FOR GOOD CLINICAL PRACTICE IN HUMAN PARTICIPANTS IN SOUTH AFRICA (2016).

vii) Netcare must be furnished with a STATUS REPORT on the progress of the study at least annually on 30th September irrespective of the date of approval from the Netcare Research Operations Committee as well as a FINAL REPORT with reference to intention to publish and probable journals for publication, on completion of the study.

viii) A copy of the research report will be provided to the Netcare Research Operations Committee once it is finally approved by the relevant primary party or tertiary institution, or once complete or if discontinued for any reason whatsoever prior to the expected completion date.

ix) Netcare has the right to implement any recommendations from the research.

x) Netcare reserves the right to withdraw the approval for research at any time during the process, should the research prove to be detrimental to the subjects/Netcare or should the researcher not comply with the conditions of approval.

xi) APPROVAL IS VALID FOR A PERIOD OF 36 MONTHS FROM DATE OF THIS LETTER OR COMPLETION OR DISCONTINUATION OF THE TRIAL, WHICHEVER IS THE FIRST.

We wish you success in your research.

Yours faithfully

6/6/2019

Prof Dion du Plessis
Full member: Netcare Research Operations Committee & Medical Practitioner evaluating research applications as per Management and Governance Policy

Shannon Nell
Chairperson: Netcare Research Operations Committee
Netcare Hospitals (Pty) Ltd
Date: 9/7/2019