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EFFECT OF HALOPERIDOL ON STRESS HORMONES AND QUALITY OF SLEEP IN INTENSIVE CARE UNIT PATIENTS WITH DELIRIUM

Murtala Muhammad Dandare

WITS UNIVERSITY

A thesis submitted to the Faculty of Health Sciences,
University of the Witwatersrand
in fulfilment of the requirements for the degree of
Doctor of Philosophy Johannesburg,
2024

DECLARATION

I, Murtala Muhammad Dandare declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Signature …….

……23……day of …October 2024……

HREC Protocol Number: M189747

DEDICATION

To my family and friends

LIST OF PUBLICATIONS

Finalised:

Dandare M, Van Eyk, A, Schmollgruber S, Van Zyl RL. Patients' perceptions of sleep versus nurses' documentation in an intensive care unit. To be submitted to Australian Journal of Critical Care

ABSTRACT

Purpose: The aim of this study describes patients and nurse's perception of sleep and follows the trends of pituitary-adrenal-hormone activities among critically ill patients and to evaluate the effect of using haloperidol in ICU patients with acute oxidative stress at an academic level one hospital. The purpose was to determine the effect of haloperidol on patients' stress hormones and oxidative stress and to evaluate benefits or disadvantages of using haloperidol in ICU patients with acute stress as well as patients' self-assessment of sleep and nurses report of patient sleep. In addition, quantify haloperidol and tramadol in plasma and urine of patients' by liquid-liquid extraction using sotalol as internal standard. The concentrations of cortisol and melatonin were also determined using ELISA-assay.

Research method: This is a prospective study with quantitative approach, with data collected at multiple points during the patients' admission.

Data collection: The setting for the study was general intensive care unit at a university-affiliated, public sector and tertiary level health care institution in Gauteng Province, Johannesburg, South Africa. Data collected included clinical profile of the delirium patients and their perception of their sleep quality and factors that promoted or retarded their sleep. A pharmacokinetic profile of haloperidol and tramadol in the patients was evaluated from blood and urine samples using HPLC analysis. Elisa assay was also carried out to determine the plasma cortisol/ melatonin level.

***Results*:** The total number of participants was 94. The overall age was 45±0.49. There were more males 48 (51.06%) than females 46 (48.94%). The average length of stay was 3.4 days (range, 1-11 days). Only 20 (21.2%) critically ill patients were on haloperidol. The SAPS II score predicted mortality rate was 30.8% as against the precise ICU mortality rate of 14%. The total sleep score mean of 47.4±16.71 for patients receiving antipsychotic or sedatives (n=83) was significantly (p=0.009) lower than the rest of the patients (55.8±8.21). A correlation analysis showed some degree of correlation (r = 0.566; $p < 0.001$) between patients' perception of sleep and nurses' record. The linearity for haloperidol and tramadol was in the range of 1-200 ng/ml, run time of 3 minutes and the lower LLOD and LLOQ for haloperidol/tramadol both in mobile phase and in plasma. The percentage recovery was within the range of 80-102%. The ELISA-assay showed high level of cortisol among AKI patients with below average sleep quality.

Conclusion: The study endorsed the use of the nurse's record of patients' sleep as reliable measure to report sleep quality in the ICU setting. The proposed method is simple, specific, linear, accurate, and precise and can be used for plasma haloperidol/tramadol detection in routine clinical practice, but not suitable for urine detection of these drugs.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic Hormone
AKI	Acute Kidney Injury
APACHE	Acute Physiological, Age, Chronic Health Evaluation
ARDS	Acute Respiratory Distress Syndrome
BBB	Blood Brain Barrier
BMI	Body Mass Index
CAM-ICU	Confusion Assessment Method for the Intensive Care Unit
CBG	Cortisol Binding Globulin
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CNS	Central Nervous System
CRH	Corticotropin-releasing Hormone
CSF	Cerebrospinal fluid
DDS	Delirium Detection Score
DHEA-S	Dehydroepiandrosterone
DNA	Deoxyribonucleic acid
DPPH	2, 2-Diphenyl-1picrylhydrazyl
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
EBV	Epstein-Barr virus
EDTA	Ethylenediamine tetraacetic acid
EEG	Electroencephalogram
ELISA	Enzyme-linked immunoassay
E/U/Cr	Electrolyte, Urea, and Creatinine
FBC	Full Blood Count
FFAs	Free Fatty Acids
GABA	Gamma-Aminobutyric Acid
GAS	General Adaptation Syndrome
GH	Growth Hormone
HDL	High Density Lipoprotein
HIV/AIDS	Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
HPA-axis	Hypothalamic-Pituitary-Adrenal axis
HPLC	High Performance Liquid Chromatography
HREC	Human Research Ethics Committee
ICDSC	Intensive Care Delirium Screening Checklist
ICU	Intensive Care Unit
ICU-AW	Intensive Care Unit-acquired weakness
IL-6	Interleukin-6
IM	Intramuscular
LC/NE	Locus Coeruleus/ Norepinephrine

LDL	Low density lipoprotein
LFT	Liver Function Test
LLOD	Lower limit of detection
LLOQ	Lower limit of quantification
LOS	Length of stay
MDA	Malondialdehyde
MDD	Major depressive disorder
MT6	6-Sulphatoxmelatonin
NA	Noradrenaline
NHLS	National Health Laboratory Service
NREM	Non Rapid Eye Movement Sleep
NSAIDS	Non-Steroidal Antinflammatory Drugs
Nu-DESC	Nursing Delirium Screening Checklist
PICS	Post Intensive Care Syndrome
PICS-F	Post Intensive Care Syndrome-Family
PSG	Polysomnography
PTSD	Post Traumatic Stress Disorder
PVN	Paraventricular Nucleus
RAS	Reticular Activating System
RCSQ	Richard-Campbell Sleep Questionnaire
REM	Rapid Eye Movement-Sleep
ROS	Reactive Oxygen Species
RRT	Renal replacement therapy
SAM	Sympathoadrenomedullary system
SAPS II	Simplified Acute Physiology Score II
SCN	Suprachiasmatic Nucleus
SNS	Sympathetic Nervous System
SWS	Slow Wave Sleep
TAGs	Triacylglycerol's
TISS-28	Therapeutic Intervention Scoring System
TNF-α	Tumour Necrosis Factor alpha
TST	Total Sleep Time
VLDL	Very Low Density Lipoprotein
WHO	World Health Organisation

CHAPTER ONE - OVERVIEW OF THE STUDY

1.1 INTRODUCTION TO THE STRESS RESPONSE

The word “stress” has been defined as a pressure or tension put on a biological system, also defined as a state of mental or emotional strain resulting from worse situations (Kim and Kim, 2023). It can also be defined as any physical situation that threatens to disrupt homeostasis, resulting in noxious emotional experience accompanied by biochemical, physiological and behavioral changes that lead to immune and endocrine system malfunction (Coker *et al*., 2018) (Figure 1.1). Stress may be described as a noun, verb or as an adjective, describing itself, being itself, and cause of itself (Jewell and Mylander, 1988). More than 100 years ago, Claude Bernard reported that the ‘*milieu interne*’ must be maintained to preserve life (Vermes and Beishuizen, 2001). Walter B. Cannon (1924) further elaborated on Bernard’s hypothesis by explaining that the maintenance of a constant internal environment depends largely on the sympathoadrenal system (Top *et al*., 2024). Canon further indicated that physical or behavioral disturbances could initiate a structured response within the organism in order to maintain ‘homeostasis’ (Vermes and Beishuizen, 2001), the latter includes, alertness, increased arousal, reduce libido and eating habit, while the former has to do with rechanneling of the energy to where it was needed most, such as central nervous system (CNS), skeletal and cardiac muscles (Dorn and Chrousos, 1993).

Hans Selye described the word stress as “response” hence the stimulus to stress response is termed the stressor (Top *et al*., 2024). The stressor may either be psychosocial or biogenic, however, most stressors are, indeed psychosocial (Daniel, 2009; Everly *et al*., 2009). Hans Selye; who is also called the father of modern endocrinology, explained that individual response to stress is determined by the individuals’ perception (Jackson *et al*., 2014). A study by Everly *et al* (2009) explained the role of cognitive processes in understanding illnesses that are stress related. Illnesses that are stressed related trigger non-specific activation of the limbic system with subsequent neuromuscular action (Fredrick *et al*., 2024). These is then followed by physiological activation of the central and peripheral component of stress response as presented in Figure 1.1. The higher center persistently sends out information to the central component of the stress response system, the periphery, and immediate environment (Chrousos and Gold, 1992). Thus the stress response system is not specific, but highly coordinated to ensure attainment of the dynamic equilibrium of the organism.

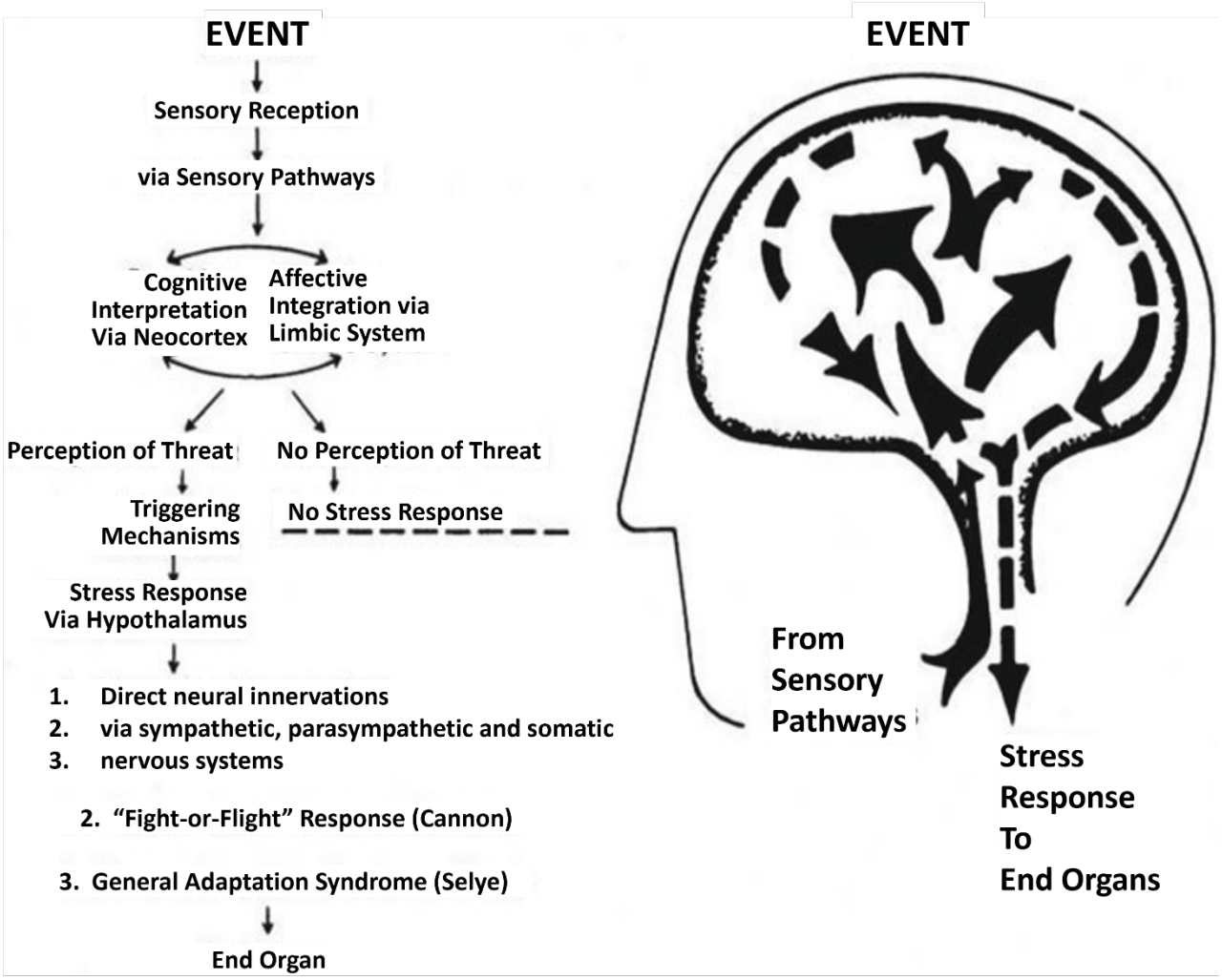


Figure 1.1: Figure pathways to stress responses (Everly *et al*., 2009).

The stressors are the triggers to stress response which resulted in metabolic and behavioral changes by putting patients at an advantage of better survival (Chrousos and Gold, 1992). Stress is one of the most important health problems in modern society and the 21st century (Kudielka and Wüst, 2010); this may further warrant research in order to identify other possible biological links between stress and health (Dresen *et al*., 2023). The hypothalamic-pituitary-adrenal (HPA) axis is the major physiological stress response system in humans (Fredrick *et al*., 2024). However, HPA-axis acclimatizes easily to various changes and is described to have higher inter-personal, intra-personal differences (Kudielka and Wüst, 2010).

In 1926, Walter Cannon, a physiologist, described the process of homeostasis, as a collaborative effort put together by the biological system to maintain equilibrium within the organism (Kim and Kim, 2024). Homeostasis was seen as an adaptation put together by the human body to maintain biological balance, which clearly augments Hans Selye's idea of understanding of

psychophysiological stress response (Rochette *et al*., 2023). Cannon *et al*., 1924 incorporated the idea of “flight or fight'' in describing the role of ANS in the stress response (Cannon, 1924). The flight or fight response prepares the body, particularly the skeletal muscles in response to threat which allow the individual to either fight or retreat from the danger (Cannon, 1953). Since then many studies described “flight or fight '' response as an “active coping” system referred to as sympathoadrenomedullary system (SAM) (Kim and Kim, 2024). In 1956 Hans Selye proposed a new theory of psycho endocrinological response termed “General Adaptation Syndrome” (GAS). He described the GAS in three phases (Rochette *et al*., 2023). The first phase is described by Selye as the “alarm” phase, describing a global somatic shock, or “call to arms'' of the body’s defense system (Rochette *et al*., 2023). Second stage is called the “stage of resistance,” characterized by depletion of the alarm stage and body response to maintain equilibrium (Rochette *et al*., 2023). Stage 1 and 2 can be ongoing throughout the lifetime; however, should the threats persist, adaptive response may be depleted, causing the body to resort to the final stage of exhaustion with obvious manifestation of symptoms of disease and dysfunction (Kazakou *et al*., 2023).

In summary, Chrousos and Gold (1992) put Cannon and Selye theories together and came up with their own definition of stress; “state of threatened homeostasis, to which the organism, preserved its internal environment, and reacts with various adaptive responses.” Subsequently (2019) new hypothesis was introduced, which stated that abnormal homeostatic response may lead to human disease, however, a good understanding of stress response may help in understanding physiological sequence of events (Chu *et al*., 2023).

1.2 BACKGROUND TO THE STUDY

Progress in critical care medicine has resulted in significant reduction in death of the patients admitted to ICU (Henderson *et al*., 2023). However, for many patients that survive ICU admission it means misery, cognitive impairment, severe life limitation and poor mental outcome (Jackson *et al*., 2009). ICU is a busy and stressful environment where patients may acquire long term psychological symptoms which may ultimately infringe on their quality of life (Scragg *et al*., 2001). All ICU patients suffer from life threatening diseases and they fall into three categories: (i) those with sudden organ abnormalities (including those that their final outcome is not clearly understood and are on prolonged organ support), (ii) those who undergoes major operation and are monitored to detect any sudden change in their hemodynamic condition, (iii) and those who their management in ICU

has failed and are receiving end-of-life care (Adhikari *et al*., 2010). The intensive care team includes not only physicians and nurses, but also other cadres like nutritionist, breathing therapist, physical and work hazards therapist, social workers and spiritual care experts (Adhikari *et al*., 2010). Patients suffering from critical illness are coping with ICU stressors which pose a great danger to their psychological wellbeing in short and long term recovery (Karaagac and Ozkaptan, 2023). Some of the outcomes include anxiety symptoms (11.9% and 43%), depression symptoms (9.8% and 30%) respectively (Scragg *et al*., 2001). However, the study was conducted at Chelsea and Westminster hospital UK. The study utilizes Hospital anxiety and depression scale (HADS), in addition to Trauma Symptom Checklist (TSC-33).

However, studies by Jackson *et al,* reported PTSD symptoms in critically ill patients can be as high as 63%, exceeding those reported in fatal medical diseases like cancer and heart attack (Jackson *et al*., 2007). In South Africa the prevalence of anxiety disorders and depression has been reported to lie between 15.8% and 9.8% respectively, and PTSD 2.3% over the lifetime period (Stein *et al*., 2008).

The stress hormone cortisol is very vital in the fight-or-flight response seen in stress ill patients, and its concentration (high or low) driven by ACTH may pose a risk of mortality in such patients (Finlay and Mckee, 1982). Critical illness is an extreme form of severe stress characterized by immediate and long-term pituitary-adrenal changes (Henderson *et al*., 2023). The acute response to extreme stress consists of structured endocrine changes aimed at generating the needed energy for fight-or-flight response with respect to alternative sources of energy for these events (Boonen and Van den Berghe, 2014). Changes in the hypothalamic-pituitary adrenal axes constitute an increase in gluconeogenesis, lipid and protein breakdown, to generate alternative source of energy for the ultimate survival of the organism, whereas the process of storage is reserve for the feature prosperous times (Boonen and Van den Berghe, 2014). With an advent of critical care medicine, many patients survive critical illnesses (Henderson *et al*., 2023). Only few of these patients escalating to chronic phase of the illness, posing a threat to the patient's recovery (Boonen and Van den Berghe, 2014). Recently many studies highlighted the mechanism and consequences of endocrine response to critical illness, hence this study intended to describe these thoughts, with particular attention to hypothalamic-pituitary-adrenal axis in relation to particular therapeutic medications and sleep quality (Showler *et al*., 2023). These resulted in patients frequently developing ICU delirium (Showler *et al*., 2023). ICU delirium can happen to both the patients and their relations and has been shown to increase death rate, chronic use of mechanical ventilation, prolonged ICU admission and cognitive impairment in future (Herling *et al*., 2018). It also comes

with the burden of cost to the patients and society in general (Showler *et al*., 2023). “Delirium is defined as the sudden brain dysfunction characterized by impairment of attention, awareness and cognition with a variable pathophysiology caused by an underlined medical disease” (Ankravs *et al*., 2023). Although delirium can be acute and reversible in nature its occurrence in the ICU may result in prolonged cognitive consequences (Arumugam *et al*., 2017). Misdiagnosis by the attending physicians may often lead to poor management of patient’s. Early detection of predisposing factors by the management team and standardized care has been shown to play a vital role in the management of delirium patients with good outcomes (Wassenaar e*t al.*, 2018). However, use of non-pharmacological options and pharmacologically based options may help the patients who develop established symptoms (Ankravs *et al*., 2023). Studies have shown that up to three-quarter of the delirium patients remained undiagnosed probably due to poor or no reliable assessment tool but recently many tools were developed and validated for a variety of ICU patients, hospital wards and emergency units (Barr *et al*., 2013a). The validated tools were formed based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 2009) criteria of the American Psychiatric association (Nydahl *et al*., 2024). Some of these tools includes: “Confusion Assessment Method (CAM-ICU), Intensive Care Delirium Screening Checklist (ICDSC), Delirium Detection Score (DDS), Nursing Delirium Screening Checklist (Nu-DESC), and Neelon and Champagne Confusion Scale, however, among all the tools, CAM-ICU was the most commonly tested followed by ICDSC” (Luetz *et al*., 2010, Immers *et al*., 2005, Devlin *et al*., 2007). The former measures the level of judgment and attentiveness among conscious patients; however, lack of these two variables qualifies the patient as having delirium (Nydahl *et al*., 2024). A prospective study was performed in 10 ICUs of Utrecht teaching and rural hospital in the Netherland, the study validated the tool among critically ill patients, with higher sensitivity and specificity than any other delirium assessment tool [64% versus 43%; 88% versus 95%] respectively (van Eijk *et al*., 2008, van Eijk *et al*., 2009).

The exact pathophysiology of delirium in critically ill patients is not fully understood, but many theories have been hypothesized (Nydahl *et al*., 2024). Some recently published studies have shown significant association between anticholinergic drugs used in the ICU and occurrence of delirium; hence favoring the cholinergic deficiency hypothesis (Hshieh *et al*., 2008, Han *et al*., 2001). Risk factors for delirium are many; however, a systematic review study identified up to 25 predisposing factors for delirium in ICU (Van Rompaey *et al*., 2008). Majority of these factors were termed precipitating factors and are caused by underlying illnesses (nine factors- are investigations based, while seven factors were related to drug treatment). The remaining four factors were termed

predisposing factors for delirium (old age, respiratory disorder, chronic alcoholism and dementia) (Wu *et al*., 2023). Other precipitating factors of delirium in ICU include: severe sepsis, cardiac arrest, in addition to many other drugs such as opiates, benzodiazepines, anticholinergic, corticosteroids, antihistamines and metoclopramide (van Munster *et al*., 2007). Drugs like midazolam and lorazepam have been tagged as independent risk factors for occurrence of delirium in ICU by some researchers (Girard *et al*., 2008). Proper management of acute illnesses and taking into consideration the predisposing risk factors may help in alleviating some of these symptoms of delirium (Wu *et al*., 2023).

Many strategies have been employed in management of delirium patients in ICU in clinical practice, including pharmacological and non-pharmacological. The non-pharmacological approach was mainly targeting the high risk elderly, and was shown to be effective by up to 40% (Zhao *et al*., 2023). These non-pharmacological measures are the use of eye glass, music, and early mobilization, prevention of sleep deprivation and correction for dehydration (Inouye *et al*., 2006). The use of earplugs before sleep has also been shown to reduce noise level, and improved sleep, especially when used two days after admission (Van Rompaey *et al*., 2012).

The use of antipsychotic (haloperidol, risperidone) is part of the management protocol in clinical practice (Carayannopoulos *et al*., 2024). However, the effectiveness of these drugs on critically ill patients with delirium has not been reported in the past (Carayannopoulos *et al*., 2024). The most frequently used antipsychotic drug in the treatment of delirium in ICU is haloperidol (75-80%) followed by atypical antipsychotic drugs 35-40% (Ely *et al*., 2004b, Patel *et al*., 2009). Haloperidol remains the drug of choice for delirium treatment in ICU and has been endorsed by the Society of Critical Care Medicine (Jacobi *et al*., 2002). The effective dose varies but can be adjusted depending on the patient's severity, and can be given at 2-10 mg intravenously every six hours (Andersen-Ranberg *et al*., 2023). Side effect of these drugs (haloperidol) are usually related to motor in balance, such as abnormal muscle tone, akathisia and dose dependent QTc elevation (Riker *et al*., 1997). It has been shown that haloperidol has the prophylactic potential of reducing 28 day mortality with good outcome among predisposed ICU patients especially when used to inform of continuous infusion in agitated delirium type (van den Boogaard *et al*., 2013). The study was conducted in a 33 bed capacity ICU at the Radboud University, Nijmegen medical center, The Netherland.

Critically ill patients also suffered from oxidative stress which formed the major pathophysiology of injury among this group of patients (Ntalapascha *et al.*, 2013). High turn-over of this reactive oxygen

species is associated with cellular injury through activation of mediators of inflammation, alteration in basic protein structure, lipids and building blocks of DNA (Di Meo *et al*., 2016). Many organ dysfunctions have been linked to the oxidative stress in critically ill patients (Dresen *et al*., 2023). Severe sepsis survivors have been reported to have excess level of free radical oxygen species activity than non-survivors (Cowley *et al*., 1996). Acute and chronic kidney disease patients were also reported to have high free radical activities (Dresen *et al*., 2023). Oxidative stress may cause both structural and functional malfunction among cardiac arrest patients with resultant reduction in cardiac cell number and reduced contractility (Dresen *et al*., 2023). Impaired oxygen utilization due to abnormal mitochondrial activities has been shown to be responsible for the degree of abnormalities among critically ill patients (Mantzarlis *et al*., 2017). Proper understanding of the oxidative stress underlying mechanism and how it relates to organ functions and regulation of the internal environment is critical (Dresen *et al*., 2023). Adequate knowledge on pathophysiology of oxidative stress and its impact on the several aspects of critical illness may also help in improving patient's management (Dresen *et al*., 2023).

Melatonin is a hormone that plays a significant role in sleep-wake cycle and is thought to play a vital role in prevention and in pharmacological management of delirium patients (Choy *et al*., 2018). The role of this hormone in the management of delirium and its antioxidant properties are still unclear (Duan *et al*., 2023). There is, however, a clear relationship between the duration of sleep and melatonin secretion which can be incorporated among critically ill ICU patients to serve as a measure into their sleep quality (Aeschbach *et al.*, 2003). The controversy put forward in the work of Torres-Farfan *et al*, was that the effect of melatonin on cortisol rhythm and sleep-wake cycle is indirectly related, and that a therapeutic dose has been reported to inhibit the ACTH- derived cortisol release (Torres-Farfan *et al*., 2003). The promising benefit of melatonin among ICU patients is generally based on its ability to induce sleep and reduce poor sleep quality experienced by ICU patients (Bourne and Mills, 2006, Kunz *et al*., 2004).

Apart from pain; sleep deprivation is a major complaint from ICU patients and it has several identifiable side effects resulting from it, both systematically and mentally (Babson *et al*., 2010). As part of the management of delirium, patients require good quality sleep, hence the two interact together (Kuhlmann *et al.,* 2023). The major etiology between the two is not fully understood (Kuhlmann *et al*., 2023). Many factors are directly related to sleep deprivation in ICU; for example, disease severity, drugs used in ICU; ICU environment and disturbance from attending staff, high

pitched sound and mechanical ventilation (Andersen *et al*., 2013). Sleep inducing drugs and painkillers like morphine, diazepam and propofol administered to reduce pain significantly decrease slow wave sleep (SWS) and Rapid Eye Movement Sleep (REM-sleep) (Andersen *et al*., 2013). Benzodiazepines are strongly associated with etiology of delirium and reduce sleep quality (Pandharipande and Ely, 2006). The current advocacy towards patients- centered care is the need for detailed qualitative assessment of sleep systematically (Tronstad et al., 2023). Achieving this aim of patient's centered care may stimulate nurses to influence patient improvement, rehabilitation and length of ICU admission (Tronstad *et al*., 2023). Polysomnography (PSG) has been identified as the most sophisticated objective method of sleep assessment because it requires more expertise and resources than any subjective methods (Oxlund *et al*., 2023). The work of Kamdar *et al.*, indicates that sleep quality could not be assessed with PSG in mechanically ventilated ICU patients (Boonen and Van den Berghe, 2014, Kamdar *et al*., 2012). However, the work of Kamdar *et al*., 2012 showed the use of "Richard-Campbell-Sleep Questionnaire to assess sleep quality among ICU patients", in addition to nurses' perception of patients' sleep and patients' reports of their own sleep (Kamdar *et al*., 2012).

1.3 PROBLEM STATEMENT

Patients who experience critical illness have elevated levels of circulating stress hormone, cortisol, with hypercortisolemia increasing carbohydrate and lipid metabolism, cardiovascular changes increasing the secretion of adrenaline and noradrenaline; as well as structured immune response. The adaptive metabolic response helps ICU patients survive the critical illness; however different patients have varying responses to acute stress. As such, patient illness severity, hematological and biochemical changes, as well as the levels of stress hormones should be monitored such that preventive and corrective measures can be implemented to reduce ICU mortality and minimize the chances of having short and long-term complications. Patients with delirium receiving haloperidol in the ICU were investigated to monitor the changes in stress hormone levels, as it has been reported to suppress CRH (Cortisol Releasing Hormone) and increase cortisol levels. Sleep quality was measured using the RCSQ (Richard Campbell Sleep Questionnaire); as well as the patient bed letters documenting sleep patterns as noted by the nurses, was also assessed.

1.4 RESEARCH QUESTION

What is the effect of using haloperidol on pituitary-adrenal hormone activities and oxidative stress in ICU patients, and how does this impact on sleep quality whilst in an ICU at an academic level hospital in Gauteng

1.5 RATIONALE OF THE STUDY

Treatment of delirium in ICU are constantly changing to optimize therapy and outcomes, as well as to ensure there is a high beneficial to harm ratio. There is sparse scientific evidence available for use of haloperidol in ICU patients with delirium, even though clinically it clearly has benefit. As such research needs to be undertaken to bridge this gap. In addition, critical illness and requisite ICU therapies expose patients to stressors, including strain on the hypothalamic-pituitary-adrenal axis that need further evaluation in the South African population.

The CAM-ICU reporting forms part of the routine monitoring of patients by ICU nurses. Inconsistent reporting, could however, lead to inaccuracy of interpretation and incorrect treatment. The increase number of ICU admissions combined with limited progress in critical care in South Africa may lead to a possible increase in mortality. However, for many patients that survive ICU admission, they usually present with severe life limitations, obstacles, cognitive dysfunction and psychological sequelae following the post discharge period that may reduce their quality of life (Jackson *et al*., 2009). Adapting to the stressors in the ICU environment and critical illness may have both long- and short-term psychological consequences that can affect both the recovery from the illness and their mental health status (Hatchett *et al*., 2010).

1.6 AIM AND OBJECTIVES

The **aim** of this study was to evaluate the effects of haloperidol in ICU patients with delirium and acute stress and the perceived quality of sleep whilst in an ICU at an academic level Gauteng hospital.

The **objectives** of this study were:

1. To determine the pituitary-adrenal-hormones and related metabolic variables in ICU patients with acute stress.
2. To determine if age and gender affect stress hormone levels.

3. To describe ICU patients' self-report assessments of sleep during their ICU stay.
4. To evaluate factors identified by ICU patients that could promote sleep.
5. To examine the relationship between the nurse's assessment of sleep and the patient's self-reported sleep.
6. To produce drug monographs on haloperidol and tramadol.
7. To establish and validate the high-performance liquid chromatography (HPLC) methodology to detect haloperidol and tramadol in plasma and urine.
8. To determine the plasma and urine levels of haloperidol and tramadol in ICU patients with delirium and acute stress.
9. To evaluate the effect of haloperidol on stress hormones (cortisol and melatonin) in ICU patients with delirium and acute stress using ELISA kits.

The objectives were achieved as described in Table 1.1.

Table 1.1: Tabular description of objectives of the study.

S/N	Objectives	Population	Data collection	Validity & Reliability	Data analysis
1.	To assess patients' confusion status, illness severity, depression and anxiety as well as post-traumatic stress.	n= 50 Include only ICU patients receiving haloperidol medication	Measuring tools included CAM- ICU, SAPS II, DASS and PTSD, to (Appendix-D)	Inclusion and exclusion criteria were followed.	Chi-square test
2.	To determine the pituitary-adrenal hormones and related metabolic variables in ICU patients with acute stress	n= 100 ICU patients receiving treatment and fulfill all the inclusion criteria	Data collection sheets were used to record all laboratory tests, including hematological & biochemical blood test (Appendix D)	A pilot study was conducted to ensure feasibility of the study. Data collection was done by the researcher.	Mann - Whitney test
3.	To determine if age and gender affect stress hormone level	n= 100 Male and female patients receiving treatment in the ICU	Patients' demographic, hematological and biochemical test variables were collected using data collection sheets (Appendix D).	This allowed for the investigation of unusual or unexpected results during data collection and investigate possible cause that may yield these results	Logistic regression (odd ratio) model
4.	To describe ICU patient's self-report assessment of sleep during their ICU stay	n = 50 Stabilized ICU patients who are able to supply answers to the questions ask by the researcher	Richard-Campbell sleep questionnaire was administered to the ICU patients with the assistance of nurses on duty. Demographic data and their quality of sleep in the ICU were assessed and compared to the results of the nurse's assessment.	A pilot study was conducted to ensure feasibility of the study. This was ensured by the researcher.	Descriptive analysis

Table 1.1 continued: Tabular description of objectives of the study.

	Objectives	Population	Data collection	Validity & Reliability	Data analysis
5.	To evaluate factors identified by ICU patients which could promote sleep	n= 50 Patients that are stable enough to give relevant answers and communicate verbally in English.	Two additional questions were added to the original questionnaire to assess patients' sleep in ICU after he/she was transferred to the ward	An appropriate sample size (n= 50) has been determined so as to be representative of the population under study.	Descriptive analysis
6.	To examine the relationship between the nurse's assessment of sleep and patient's self-reported sleep	n= 50 ICU patients receiving haloperidol and tramadol medication	Information regarding patients' sleep as recorded by the nurses in the record folder was obtained and compared with the patient's self-reported sleep.	An appropriate sample size (n= 50) has been determined so as to be representative of the population under study.	Descriptive analysis
7.	To produce drug monographs on haloperidol and tramadol.	Not applicable	Information collected from textbooks and publications from the following databases: CINAHL, MEDLINE/PubMed, SCOPUS, and a search engine like Google scholar. The following keywords for searching relevant literature were included: Acute stress, Haloperidol, critically ill, quality sleep, pituitary-adrenal hormones.	A wide search of all available information was collated and verified.	Descriptive analysis

Table 1.1 continued: Tabular description of objectives of the study

8.	8. To establish and validate the high-performance liquid chromatography (HPLC) methodology to detect haloperidol and tramadol in plasma and urine.	nil	Laboratory experiments were conducted to establish and validate the high-performance liquid chromatography (HPLC) methodology to detect haloperidol and tramadol in plasma and urine.	This ensured consistency and accurate recording of data. An appropriate sample size (n= 50) has been determined so as to be representative of the population under study. The reliability of the measuring tools was ensured by Cronbach's alpha, and values of 0.7 were considered as reliable.	Chi-square test
9.	To determine the plasma and urine levels of haloperidol and tramadol in ICU patients with delirium and acute stress.	n= 50 Included only ICU patients receiving haloperidol and tramadol medication	Used validated HPLC methodology to detect haloperidol and tramadol in plasma and urine.	This ensured consistency and accurate recording of data.	Chi-square test
10.	To evaluate the effect of haloperidol on stress hormones (cortisol and melatonin) in ICU patients with delirium and acute stress using ELISA kits.	n= 50 Include only ICU patients receiving haloperidol medication	Cortisol and Melatonin levels were determined using standardized ELISA-kits.	This ensured consistency and accurate recording of data.	Chi-square test

CHAPTER TWO (2a) LITERATURE REVIEW

2.1 STRESS RESPONSE TO ACUTE STRESSORS

For the coordinated stress responses, following psychophysiological stimuli, consist of three pathways being neural, the neuroendocrine, and the endocrine axis respectively (Kazakou *et al.*, 2023). During stress arousal the three main components of neural axis namely, the sympathetic, parasympathetic as well as the neuromuscular nervous system ensure a rapid and quick response to the target organs (Kazakou *et al.,* 2023). The neuroendocrine component ensures the concept of "flight or fight," model whereby the organism ensures a coordinated response against the threat or retreat from the threat (Kazakou *et al*., 2023). The last component is the endocrine axis, which is the most prolonged somatic stress response (Mason, 1968).

The hypothalamic-pituitary-adrenal-axis is the main endocrine stress axis in humans (Leistner *et al*., 2020). Following acute stress, cells confined to the paraventricular nucleus (PVN) in the hypothalamus mediate the release of Corticotropin Releasing Hormone (CRH), and stimulate the pituitary gland to release Adrenocorticotropic Hormone (ACTH) into the bloodstream (Zorn *et al*., 2017) (Figure 1). At the level of adrenal cortex, ACTH mediates the release of cortisol into the blood circulation (Leistner *et al*., 2020). The release of cortisol initiates a wide range of effects on metabolism, cardiovascular and immune responses (Rahardjo, 2015). During critical illness, there is reduced cortisol breakdown and elimination resulting in hypercortisolemia, probably due to reduced action of cortisol-metabolizing enzymes secondary to ACTH suppression (Peeters *et al*., 2017). Some studies show that lipid depletion and reduced ACTH regulated gene expression may result in adrenal insufficiency in long-stay ICU patients (Boonen *et al*., 2013). This observation was confirmed by studies that indicated that administration of exogenous corticosteroids may shorten the duration of ICU stay (Annane, 2016). The complex interaction of the various stress hormones affected during an acute illness may negatively affect adrenocortical function in the prolonged phase of critical illness (Leistner *et al*., 2023). As such appropriate management during the acute phase of critical illness could have long-term benefits on these patients (Leistner *et al.,* 2023).

The hallmark of the stress system is thus the regulation of the adaptive responses of the biological system to various stressors (Stratakis and Chrousos, 1995). One of the important parts of the stress system is CRH and locus Coeruleus norepinephrine systems (LC/ NE) which work hand in hand to

regulate each other's activity, so that stimulation of one promotes the activation of the other and vice versa (Kazakou *et al*., 2023). The stress system is therefore self-regulated with negative feedback, exerted on stress mediator release, both peripherally and centrally (Michelson *et al.*, 1995), which persistently threatened by stressors. Activation of the stress response by stressors can lead to dysphoria, somatic disease and emotional disturbance (Stratakis and Chrousos, 1995).

2.2 THE EFFECT OF ACUTE AND CHRONIC STRESSORS ON PITUITARY- ADRENAL HORMONES

2.2.1 Introduction

The acute stressors are counteracted through the action of catecholamines and cortisol, but when the stress becomes persistent (physical or emotional) the individual adaptive responses become stereotypic described by Selye as "The General Adaptation Syndrome." (Mbiydzenyu and Qulu, 2024) However, prolonged exposure to stress, both the HPA-axis and LC/ noradrenaline system become chronically activated (Mbiydzenyu and Qulu, 2024). The individual becomes more alert, and the brain focuses more to the threat, resulting in increased heart and respiratory rate (Kim and Kim, 2023). This results in accelerated breakdown of biological building blocks, and blood flow is channeled towards the alerted brain, heart and skeletal muscles, to enable the individual flee or fight (Kim and Kim, 2023). It has been shown that stress (inflammatory, physical, and emotional) is associated with simultaneous activation of the HPA (Stratakis and Chrousos, 1995). Cytokines and other mediators of inflammation also stimulate the stress system (Chu *et al.,* 2024). TNF-α, interleukin 1β and interleukin-6 can initiate activation of HPA as a single entity or in combination with each other mediators (Chu *et al*., 2024). The IL-6 does so more often in chronic inflammatory stress and is considered to have both pro- and anti- inflammatory properties (Steptoe *et al*., 2007). Chronic activation eventually leads to tissue damage and receptor desensitization, due to the increased release of inflammatory mediators (Sapolsky *et al*., 2000).

2.2.2 ACTH

ACTH is a 39 amino acid peptide produced in the pituitary from the larger molecule, proopiomelanocortin (Moch *et al*., 2005). ACTH stimulates the adrenal-cortical secretion of glucocorticoids so that circulating concentration of cortisol is increased (Moch *et al*., 2005).

Maintenance of adequate level response of HPA-axis during chronic stress is important for survival (Ahrorbek *et al*., 2023). Three patterns of response are seen depending on the type of stress; (i) desensitization of ACTH response to stimulus, (ii) no desensitization of ACTH with hyper-responsiveness as well as (iii) small and transient increase in ACTH, with sustained increase of serum corticosterone and reduced ACTH response (Aguilera, 1994). Gold *et al*., 1995 reported that major depressive disorder (MDD) and Cushing's disease shows similar levels of hypercortisolism, due to a defect at or above the level of hypothalamus, resulting in the hyper-secretion of CRH (Gold *et al*., 1995). Sapolsky *et al.* (2000) have shown that, hypercortisolism in a rat model can damage hippocampal cells that contain glucocorticoid receptors that mediate cortisol induced suppression of the CRH neurons (Sapolsky *et al.*, 2000). In humans, during a CRH response test in patients with MDD, PTSD and anxiety, shows poor sensitivity of ACTH to CRH (Gold *et al*., 1988). In animal models AVP was reported to be the main driver of HPA-axis (Scaccianoce *et al*., 1991). Human patients with chronic stress show hypercortisolism more so than compared to normal cohorts (Ahrorbek *et al*., 2023). These patients also demonstrated poor response of ACTH to AVP (Ahrorbek *et al*., 2023). This result is consistent with the notion that HPA-axis is driven by AVP in chronic stress (Michelson *et al*., 1994). In the work of Fink, 2000 the shift from CRH to AVP as the driver of ACTH release in chronic stress, was attributed to distorted feedback response and depleted glucocorticoid receptors during chronic stress (Fink, 2000).

2.2.3 Cortisol

Cortisol is a glucocorticoid hormone secreted by the outer cortex of the adrenal gland (Thau and Sharma, 2023). CRH and ACTH stimulate cortisol secretion through a (negative) feedback mechanism (Gatti *et al*., 2009). The hormone plays a vital role in the regulation of most important physiologic processes, including energy metabolism, electrolyte balance, blood pressure control, immune modulation and stress response, cell proliferation and differentiation, as well as memory and cognitive function regulations (Saiah, 2008). The major portion of the drug exist bound to the plasma protein corticosteroid binding globulin (CBG or transcortin) and to albumin, only 3-5% of the total plasma cortisol circulate in the unbound free form, which is the active form (Torpy *et al.*, 2004). Cortisol is secreted predominantly in the urine as glucosiduronates and sulfates, generated primarily in the liver with reduction of A-ring to form tetrahydrocortisol and allo-tetrahydrocortisol catalyze by α and β reductases and then by 3α and 3β-hydroxyl-steroid dehydrogenase. Plasma cortisol levels are characterized by circadian rhythm with a morning maximum, declining levels throughout the

daytime, a period of low concentration around midnight and a rise after a few hours of sleep (Van Cauter *et al*., 1996). The circadian rhythm is linked to sleep-wake cycle and light-dark cycle; the circadian rhythm is more recognized with plasma cortisol, than in urine or saliva cortisol, but this notion is currently contradicted by some researchers (Morineau *et al*., 1997).

Glucocorticoid action can be influenced at various levels, based on its ability to bind to glucocorticoids receptors, thus changing the receptor sensitivity and modulating HPA-axis activities (Draper and Stewart, 2005). At pre-receptor level, 11β-hydroxysteroid dehydrogenase 1 (11BHSD 1) facilitate glucocorticoid exchange by converting cortisone to cortisol to aid glucocorticoid receptor action (Draper and Stewart, 2005). Intracellular receptor type 1, mineralocorticoid receptor is highly sensitive to cortisol and aldosterone, while type II glucocorticoid receptor has greater affinity to synthetic steroids, as example, dexamethasone and low affinity to endogenous hormone (Mao and Chen *et al*., 2023). At post receptor level both glucocorticoid receptor and mineralocorticoid receptor mediate the effect of hormone by translocation to the nucleus binding to the specific DNA and modulate mRNA (Draper and Stewart, 2005). In studies by Kalin *et al*., Chrousos and Gold, 1992 testing the HPA-axis feedback, 1 mg of dexamethasone was administered to healthy people, they showed marked suppression of ACTH and cortisol secretion (enhanced glucocorticoid receptor negative feedback) (Mao and Chen, 2023). However, in chronic stress patients the 1 mg dexamethasone failed to suppress cortisol secretion whereby indicating hyperactivity of the HPA-axis during stress (Kalin *et al*., 1982, Chrousos and Gold, 1992, Kathol *et al*., 1989).

2.2.4 Growth hormone

Growth hormone is a protein, containing 191 amino acid sequence, secreted from the anterior pituitary (Halmos *et al*., 2023). The release of this hormone is mediated by growth hormone-releasing factors from the hypothalamus (Casanueva, 1992). Growth hormone is also known as somatotrophin, and plays a major role in growth regulation, particularly in the perinatal period and in childhood (Halmos *et al*., 2023). The role of growth hormone in stress is not fully understood than that of the adrenal cortical axis (Halmos *et al*., 2023). However, many studies have documented its release during response to psychological stimuli in humans (Selye, 2013). Selye *et al* (1956) also reported that GH causes the release of mineralocorticoids. Growth hormone action is mainly mediated through small protein hormones called insulin-like growth factor (ILGFs) produced in the

muscle, liver and other growth hormone -sensitive tissues (Casanueva, 1992). Yuwiler *et al* (1976) reported that growth hormone has a diabetic-like insulin-resistant effect, whereby inhibiting glucose uptake, promoting lipolysis, stimulating protein synthesis and inhibit protein breakdown. Growth hormone secretion has also been shown to increase in response to surgery and trauma, in relation to the severity of the injury (Casanueva, 1992). Chronic treatment with glucocorticoid has been reported to suppress growth hormone secretion and impair growth, for example emotionally deprived children were shown to have stunted growth but ultimately normalize after removing them from the threatened environment (Allen, 1996, Albanese *et al*., 1994).

2.2.5 Prolactin

Prolactin hormone is pleiotropic protein hormone containing 199 amino acids, produced and released from the cells of anterior pituitary, the lactotrophs, with a structure similar to that of growth hormone (Jaroenporn *et al*., 2007). The hormone secretion and release is heightened during pregnancy and stimulates milk secretion (Casanueva, 1992). Prolactin has shown strong responsiveness to psychological stimulation but its role in disease conditions has not been well established (Makara *et al*., 1980). The work done by Chang et al., and Kaminska et al showed that the hormone can stimulate corticosterone release from the adrenal gland with many of its receptors expressed in several species (Chang *et al*., 1999, Kaminska *et al*., 2002). Also, work done by Dijksttra and Tohei *et al.,* it was in addition to the HPA-axis stimulation, prolactin release also occurs during stress response in rats (Dijkstra *et al*., 1992, Tohei *et al.*, 1997). Exposure to endotoxin, pro- inflammatory markers like IL-1 and IL-6 has been reported to stimulate prolactin release with inconsistency with other studies that reported contradictory action of prolactin hormone (Gala, 1990, Taylor *et al*., 1995).

2.2.6 Dehydroepiandrosterone-sulphate

DHEA is an ACTH-regulated C-19 steroid synthesized from cholesterol and classified with the adrenal androgens, androstenedione, and testosterone (Baulieu *et al*., 2000). The serum concentration of dehydroepiandrosterone (DHEA) and DHEA-sulphate, are decreased in critically ill-patients and are the most common steroid secreted by the adrenals under pituitary ACTH control alongside cortisol (Vermes and Beishuizen, 2001). Some portions of DHEA and DHEA-S are produced in the CNS and its physiological role is not well understood, but there is clear indication that it is a general parameter of well-being and a potent modulator of the immune response (Vermes and Beishuizen,

2001). The work of Kalimi and Lardy et al data reveal the role of DHEA biochemical/ physiological role in various cell types, tissues, and organs (Kalimi *et al*., 1994, Lardy and Stratman, 1989). As such DHEA has shown to have effects in stress, obesity, aging, carcinomas, diabetes, pregnancy, cardiovascular and nervous system pathophysiology (Nenezic *et al*., 2023). Physiologically, the serum concentration of DHEA (S) oscillates parallel to that of cortisol but a remarkable dissociation between serum cortisol and DHEA (S) concentration can be observed during severe illness (Vermes and Beishuizen, 2001). DHEA-S is abundant in plasma and can be easily measured compared to DHEA (Nenezic *et al*., 2023), it also has a long half- life with no diurnal variation (Hornsby, 1995). Kalimi et al (1994) reported antiglucocorticoid and antidepressant effects of DHEA-S and DHEA. Morgan et al (2004) conducted a study among healthy volunteers in military school and reported increased DHEA-S level in response to acute stress (Morgan *et al*., 2004). The work however, suggested that DHEA-S may confer psychological and neurotrophic effects during acute stress and that DHEA/ cortisol ratio may explain the extent of resilience to the negative effect of a stressor (Nenezic *et al*., 2023).

2.2.7 Aldosterone

Aldosterone is a hormone secreted from the cells of the zona glomerulosa of the adrenal cortex, and its secretion is under multifactorial control of hormones, such as angiotensin II, ACTH and potassium ions as well as an inhibitor, like atrial-natriuretic (Fraser et al., 1979). Aldosterone acts via mineralocorticoid receptors to promote transepithelial sodium transport (Vecchiola *et al*., 2024). Fraser et al., 1997 reported that administration of ACTH or stress-mediated ACTH release in normal people elevates plasma aldosterone level. Plasma aldosterone has been shown to fluctuate synchronously with plasma concentration of cortisol; this shows evidence that ACTH functions in minutes-to-minutes control of aldosterone secretion (Weinberger et al., 1975). It has also been shown that dexamethasone administration suppresses the rhythmic action of cortisol but those of aldosterone persist (KATZ *et al*., 1975) suggesting that the source of aldosterone, ACTH rhythm are the same but that of aldosterone is not mediated by ACTH. Moreover, normal people show lack of aldosterone- cortisol synchrony (James *et al*., 1976). Meanwhile, psychological or physical stressors that elevate ACTH and catecholamine release do not cause strong or sustained elevations in aldosterone level but chronic fluid loss such as diarrhoea, and sweating, raise aldosterone level significantly (Vecchiola *et al*., 2024).

2.3 STRESS AND METABOLISM

Acute activation of HPA-axis results in adaptive metabolic changes that prepare the individual by providing a source of energy in order to increase its chances of survival (Rabasa and Suzanne, 2016). Some of the changes include elevation of serum glucose, glycogenolysis, gluconeogenesis and breakdown of triglycerides into free fatty acid and glycerol, insulin resistance and hypertension (Rabasa and Suzanne, 2016). These metabolic changes play a vital role in providing short-term energy to the organism; however, persistence of these threats predisposes the organism to cardiovascular morbidity and mortality as well as metabolic diseases like diabetes mellitus (Masenga *et al*., 2023).

2.3.1 Insulin

Glucocorticoids and catecholamine mediate the effects of insulin concentration during prolonged stress (Vali *et al*., 2024). Chronic stress leads to HPA-axis activation that may cause insulin resistance, reduced insulin release from the β-cells of the pancreas, increase production of glucose through gluconeogenesis, glycogenolysis by increasing glucagon secretion, and lipid breakdown, resulting in hyperglycemia and hypercholesterolemia (McEwen, 2001).

2.3.2 Carbohydrate

Glucocorticoids affect the glucose metabolism through synthesis and stimulation of specific enzyme, (3^1, 5^1, cyclic AMP) which may ultimately result in hyperglycemia (through stimulation of hepatic gluconeogenesis, glycogen deposition and reduce peripheral glucose utilization), resulting in negative nitrogen balance and loss of fat (Baxter and Forsham, 1972). This hyperglycemia was shown to be protective, by providing sufficient energy for the stressful organism to either respond or retreat from the stressful situation (Chandel, 2021). Insulin antagonizes the effect of glucocorticoids during hyperglycemia by reversing some of the metabolic changes. Hypercortisolism has been reported to resemble Metabolic X syndrome (MS-X) in both biochemical and somatic aspect, with significant reduction in glucose utilization by cells (Taskinen *et al*., 1983, Reaven, 1988) this is characterized by poor glycemic control in diabetic patients and increase urinary free cortisol excretion (Chandel, 2021).

Stress hyperglycaemia in diabetic patients result from relative insulin deficiency, coupled with β-cell

secretory defect (Chandel, 2021). The manifestation of stress hyperglycemia is probably due to complex process that involves counter-regulatory hormones such as, growth hormone, cortisol, epinephrine and norepinephrine, resulting hyperglycemia and peripheral insulin resistance (Preiser, 2012, Lena *et al*., 2011).

2.3.3 Cholesterol

Under normal condition or physical activity, excess food is stored within adipose tissue in the form of triglycerides but during stress, the latter are broken down into free fatty acids (FAs) and glycerol, and later released into the systemic circulation (Peckett *et al*., 2011), to help supply the organism's increase energy demands (Saher, 2023). Chronic stress increase total cholesterol level and low density lipoprotein (LDL) (Saher, 2023). These changes had been suggested by Brindley *et al*.1993 that results from stress-induced sedentary lifestyle or uncontrolled diet (Saher, 2023). However, adrenaline release, initiates very low density lipoprotein (VLDL) breakdown that results in availability of circulating LDL (Brindley *et al*., 1993). Glucocorticoids are responsible for the increase activity of lipoprotein lipase enzyme through increase in transcription or posttranslational modification (increase activation or decrease degradation) that play a vital role in hydrolyzing TAGs to make FFAs available to the tissues for use or storage depending on the circumstance (Ottosson *et al*., 1994).

2.3.4 Triglycerides

Body triglycerides (TGs) are stored in the adipose tissue while the synthesized triglycerides are secreted from chylomicrons and other lipoprotein (Gugliucci, 2023). These TGs are broken down together to release free fatty acid (FFA) and glycerol in a blood circulation (Iapichino *et al*., 2006, Krogh- Madsen *et al.*, 2008). However, production of lipid peroxides increases significantly during the acute phase of stress response with on-going organ damage (Wilmore, 2000, Hoffer and Bistrian, 2013). Similarly, FFAs oxidation increases in the tissues and in the hepatic cells with concomitant conversion of excess FFAs into ketones or further hydrolyzed to TGs and released into the blood circulation as VLDL (Gugliucci, 2023). Also, lipid metabolism is said to have increased overall, while cholesterol metabolism and production are impaired probably due to insulin regulatory effect on TGs (Björntorp, 1996).

2.3.5 Protein breakdown and BMI

Under normal temperature and pressure, protein undergoes constant breakdown and replaced in a well-controlled and balanced process (Simonson *et al*., 2020). However, following stress the rate of breakdown of protein exceeds the rate of synthesis, influenced by inflammatory markers and some hormones (Simonson *et al*., 2020). Protein synthesis is characterized by exaggerated activation of the proteasome pathway, which results in massive breakdown of protein and the ultimate loss of muscle bulk (Simonson *et al*., 2020). The nitrogen balance is usually regarded as negative, due to the imbalance or difference between rate of degradation and synthesis (Simonson *et al*., 2020). A study by Hill et al., conducted on body composition, reported that about 5% of body mass index can be lost daily (Hill, 1992, Pichard *et al*., 2004). The result is the muscle wasting seen in the ICU-acquired weakness. A study by Herridge et al., among survivors of chronic respiratory syndrome reported chronic wasting up to five years after ICU discharge (Herridge *et al.*, 2011).

Body mass index (BMI) is a measuring scale, used to measure weight status of people (Bray, 2023). BMI is obtained by dividing the weight of an individual in kilograms by the square of his height in meters (Bray, 2023). Changes in body composition triggered by stress are usually seen during critical illness, including the fall in BMI, and the relative preservation of fat tissues (Marques and Langouche, 2013, Pichard *et al*., 2004). Also reported in a study by Sapolsky *et al*, presented that short time stress induced exaggerated appetite, while chronic stress suppressed appetite (Bray, 2023). These factors are related to brief outburst of CRH release, followed by chronic cortisol action (Sapolsky *et al.*, 2000).

2.4 CATECHOLAMINES

In 1939 W.B Cannon, described adrenaline as the main active adrenal gland hormone and the neurotransmitter of the sympathetic nervous system (Cannon and Lissak, 1939). These hormones are derived from amino acid tyrosine by axon terminals, brain and adrenal medullary chromaffin cells (Motiejunaite *et al*., 2021). Adrenaline and noradrenaline synthesis and release is controlled by a wide range of hormones and active processes through feedback mechanism, acetylcholine, prostaglandins, vasoactive amines and peptidases (Hoffman and Lefkowitz, 1996). Upon danger, the SNS is accelerated with increased activation of catecholamine (Motiejunaite *et al*., 2021). The initial response is increased heart rate, sweating, wide pupils and increased GIT motility with the hope of

eliminating the source of danger or reducing the tension (De Bellis *et al*., 1999). There is shunting of blood from less vital places to where it is needed most (skeletal muscles, heart) all with the aim of supporting the coping mechanism (Kruk *et al*., 2020). Catecholamines can be measured by assaying blood samples during acute stress because they disappear within minutes after their action; as such they were considered to have very short half-life (Berne and Levy, 1983, Ziegler, 1989).

2.4.1 Adrenaline

Once it is released from adrenal glands, adrenaline stimulates α-and-β-adrenergic receptors (Liang *et al*., 2020). The β-receptors are abundant in heart muscles; its stimulation causes an increase in cardiac output by increasing cardiac contractility and heart rate (Liang *et al*., 2020). Adrenaline also acts on smooth muscles through adrenergic receptors where it causes the redirection of blood from skin, kidneys to where it is required, such as the heart, brain and skeletal muscles (Rashidovna *et al*., 2023). Adrenaline reduces clotting time by increasing platelet adhesion (Rashidovna *et al*., 2023). Adrenaline also causes high serum glucose, by glycogenolysis and gluconeogenesis by glucagon in the liver, lipolysis is also increased by adrenaline to create alternative sources of energy. Chronic release of adrenaline into the bloodstream was associated with increased risk of CVS disease (Chrousos and Gold, 1998).

2.4.2 Noradrenaline

Noradrenaline (NA), like adrenaline, is synthesized in the brain (chromaffin cells, sympathetic nerves and ganglia) from the amino acid tyrosine (Glavin, 1985). Noradrenaline is cleared rapidly from plasma circulation by uptake into sympathetic postganglionic neurons where they are packaged and stored, or into extraneuronal cells, where they are metabolized (Goldstein *et al*., 1983). Together with adrenaline they trigger hemodynamic and metabolic actions that range from increased glycogenolysis and blood pressure. They also have catabolic action on carbohydrate, lipid, and protein and indirectly through alteration in the secretion of hormones such as insulin (Cryer, 1980). A survey done by Galvin et al, reported that noradrenaline is more or less a locally released transmitter rather than a hormone, however, is reserved to be released in an emergency state like severe stress (Glavin, 1985). In the animal study model, Glavin *et al* (1984) shows that acute stressors increase the rate of production, release of noradrenaline as well as metabolism in both CNS and nerve terminals, this explains significant reduction in the circulation.

In humans, noradrenaline aids the process of planning and execution of important behaviors

needed for life existence (Svensson, 1987). It is further reported by Charney et al, that severe stressors could elicit exaggerated response of the LC/noradrenaline activation, as applicable in animal models, and that further exposure to stressors could result in prolonged symptoms of anxiety (Charney *et al.*, 1995).

2.5 ACUTE STRESS AND THE CRITICALLY ILL

2.5.1 Acute stress

Acute stress is defined as a short acting stress, whereas chronic stress is longer acting stress and often difficult to resolve (Giglberger *et al*., 2023). For the purpose of this study, acute stress refers to the "metabolic response to stress, which is part of the adaptive response that all ICU patients experience in order to survive critical illness" (Preiser *et al.*, 2014). There are three overlapping temporal stages by which the endocrine and immune system-protect the organism by responding to internal and external stress, the stage of alarm reaction, stage of resistance and the stage of exhaustion (Perdrizet, 1997). On the other hand, anxiety is an emotional state characterized by feeling tension, experiencing worried thoughts and physical changes, as well as recurring unwanted thoughts (APA, 2017). "Critical-illness, an example of severe physical stress, is characterized by elevated levels of circulating stress hormone, cortisol" (Peeters *et al*., 2017) (Figure 2.1).

2.5.2 ICU Environment

DeMeyer *et al.,* (1967) during her interview with 24 ICU patients gathered that "Patients in ICU are receiving physical over stimulation and emotional deprivation and effort should be made to create an environment that will contribute to, rather than detract from, the physical, mental, and emotional functioning of both the patient and the staff (DeMeyer, 1967). The ICU is a unique geographical environment in which extreme levels of nursing care, organized monitoring and organ support can be provided to improve patient morbidity and mortality (Chillimuntha *et al*., 2023). However, effective intensive care requires prevention, immediate detection of warning signs and proper response through an integrated disciplinary approach before and during ICU admission, as well as comprehensive follow-up or proper pain management care (Cairns and Faulds, 2018). Critically ill-patient suffer from life-threatening conditions, therefore the aim of a patient's intensive care management includes improving the patient's physiology, the provision of organ support machines, and early detection and treatment of underlying pathology. This is achieved through an

integrated disciplinary approach, including the admitting team, and the specialized critical care team, supervised by critical care physician (Cairns and Faulds, 2018).

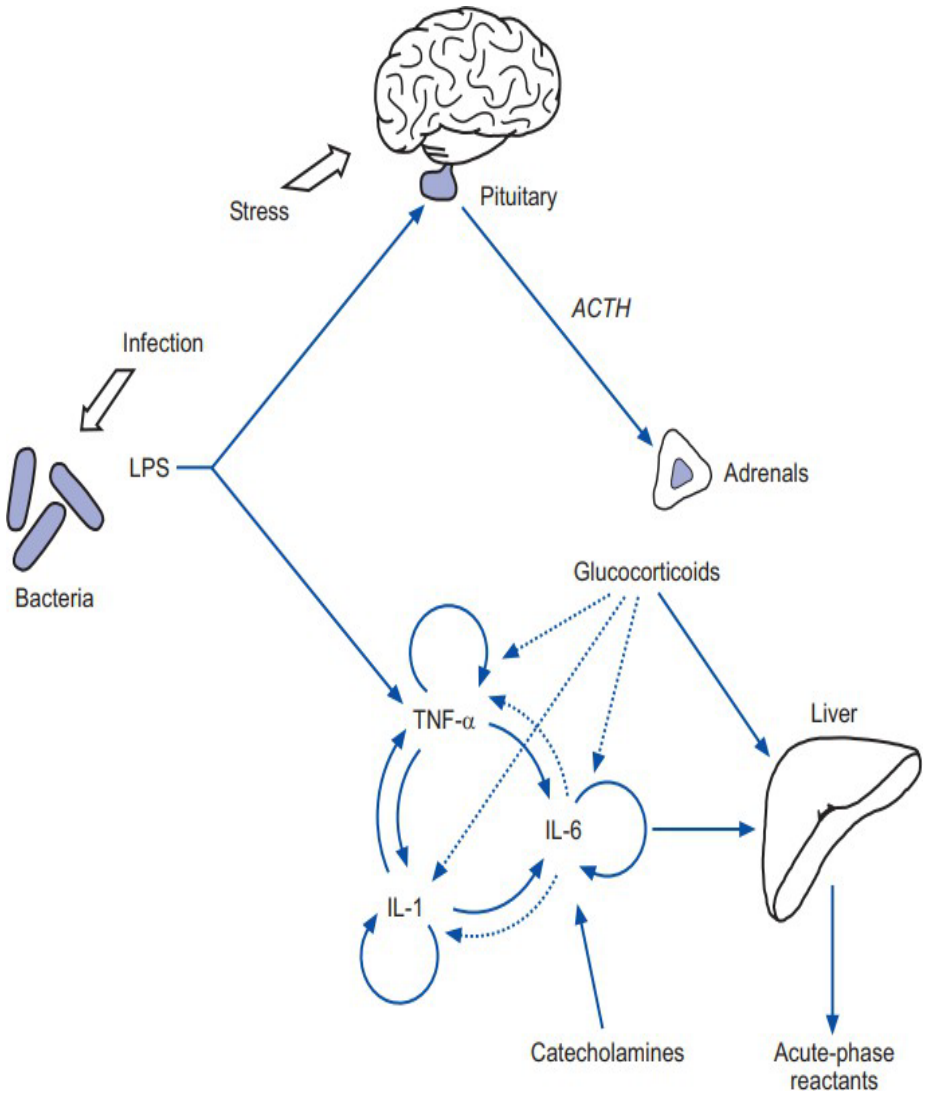


Figure 2.1: Interaction between the HPA-axis and inflammatory cytokines. LPS = lipopolysaccharide, TNF-α: Tumour necrosis factor alpha; IL: interleukin (Vermes and Beishuizen 1999).

Studies Herridge et al., 2011, reported emotional stress undergone by the ICU patients as "Torture" probably due to emotional stress experienced by these patients, however, patients (ref). However, patients with severe acute respiratory distress syndrome (ARDS), respiratory function impairment occur 2-5 years after ICU discharge, with chronic functional status impairment exceeding five years (Herridge *et al* 2011). A study by Rattray *et al*, reported that many patients are now surviving critical illness because of the availability of life supporting equipment and therapy in many hospitals compared to the previous years, which would have previously meant death (Rattray *et al.,* 2005).

However, the trauma caused by critical illness itself plus the ICU admission experience together with uncertain recovery from the disease has been associated with acute and chronic psychological sequelae (Wade *et al*., 2014). Acute kidney injury (AKI) patients treated for renal replacement therapy (RRT) are known to have poor outcomes (Neyra *et al*., 2023). Among this group an overall 47% mortality was reported, with up to 80% mortality rate at 10 years (Schiffl and Lang, 2012). A study by Scragg et al, reported depressive symptoms to be 9.8% and 30% while anxiety symptoms to be between 11.9% and 43% (Scragg *et al*., 2001). Symptoms of PTSD could be as high as 63% as shown among severe diseases like cancer and heart attack (Jackson *et al*., 2009).

2.6 ICU ORGANISATION AND ACTIVITIES

ICU is a dynamic, sophisticated working environment that involves interdisciplinary professionals (Tronstad *et al*., 2023). The environment also allows for sharing of expertise and availability of modern equipment to improve patient management to the highest level (Marshall *et al*., 2017). ICU is the recommended environment for the management of critically-ill patients especially those that have life-threatening disease and the structure involves collaboration from different professional teams (Ferri *et al*., 2015). The patients in this environment who are critically ill, require a multidisciplinary approach to treatment, implementation and constantly reviewed for maximum benefit to the patients (Ferri *et al*., 2015).

Approach to ICU care was started in the 1950s and was introduced based on the idea that critically ill patients were confined in a relatively small unit with highly skilled professionals and state of the art equipment (Vincent, 2013, West *et al*., 2014). Due to the complexity of ICU patients admitted in this environment and rapid intervention from the caregivers, theenvir onment should be set up as close as possible to the vital units of the hospital with limited distance travel by the staff, availability of the vital equipment and limited access to public (Thompson *et al.,* 2012). With good management most of the critically-ill patients can regain normal health (Crawford *et al*., 2023). This depends on the skill of the staff, and modern life support equipment (Valentin, 2011, West *et al*., 2014). Not every patient should be admitted to the ICU, but only those with reversible diseases condition (Crawford *et al*., 2023). The ICU staff should only admit those that fulfill the national guidelines and legislative documents of the country (Valentin, 2011).

Different countries have different ICU settings/ structure, and even within the same country's health

system, probably based on government priorities, resources and regulatory requirements (Marshall *et al*., 2017, Valentin, 2011). A conventional ICU should have approximately a six bed capacity with a maximum of 12 beds. This can be modified, based on the needs of the patients (Valentin, 2011). Specialized ICU's are characterized by limited number of beds (6-8 beds), hence specialist based ICU enhance consultation of the patients, unlike a general ICU, where different patients are combined in the same environment (Iwashyna *et al*., 2009).

Hence different ICU models exist, based on how they are defined or categorized (Chittawatanarat and Pamorsinlapathum *et al*., 2011). "Open" ICU, where the specialist determines the patient's admission and discharge (Chittawatanarat and Pamorsinlapathum *et al*., 2011). In the "closed" ICU design, patients are put under the care of intensivists who provide special care for critically-ill patients with good outcome (Rothschild, 2001). However, intensivists has been shown to manage patients better, due to the integrated approach, including better understanding of disease pathology, evidence based approach to the patients as well as strict compliance to the protocol (Chowdhury and Duggal, 2017). A further model, known as the "intensivists" model, where in an open ICU, every patient's receive mandatory consultation, with other doctors co-attending (Rothschild, 2001). This model enhances communication among the various teams and best-practice compliance (Breslow, 2007). The 'closed' ICU is termed to be the best as it reduced death and number of days spent in the hospital (Vahedian-Azimi *et al*., 2021). However, its demerit is that it consumes money because of the high cost of equipment's. This is a problem to most low economic countries (Treggiari *et al*., 2007, van der Sluis *et al*., 2011, Murthy and Adhikari, 2013). A semi-closed ICU may replace the 'close ICU' in terms of LOS (Length of Stay), mortality and cost (Chowdhury and Duggal, 2017).

Patient surveillance is a key mechanism in linking ICU staff and ratio-to-patient outcome for prevention of poor outcome (Khan *et al*., 2023). The process of surveillance, allows ICU staff to acquire vital information from the patients that will enable them to make good interventions (Henneman *et al*., 2010). In advanced nations the chances of survival from critical care depend largely on the availability of skilled staff, good infrastructure, and advanced life support equipment which often would not available in low income countries (Murthy and Adhikari, 2013, Rosman *et al*., 2013). Generally a higher staff: patients ratio has been reported in ICU's in high-income countries, with a mean nurse to patient ratio of above two and a physiotherapist to patient ratio of above seven (Nydahl *et al*., 2014). Regular assessment of staff adequacy will improve overall quality of care in ICU, and boost staff morale (Ward *et al*., 2013, Capell *et al*., 2019). Notably, the lack of

professional staff, poor infrastructure and lack of technologically advanced equipment in the majority of African countries makes their ICU ineffective (Murthy and Adhikari, 2013, Baker, 2009).

Recently, there is an anticipated increase in critical-illness in low-and middle income- countries on account of urbanization (Falk, 2023). The management of these patients is further complicated by a shortage of specialists, shortage of ICU beds, inadequate monitoring devices (Falk, 2023). The presence of comorbidities, such as high blood pressure, metabolic diseases like diabetes mellitus, compromise the patient response to treatment and results in prolonged LOS in ICU (Hawn *et al*., 2011, Khullar and Maa, 2012, Jerome *et al*., 2017, Ramesh *et al*., 2014, Tran *et al*., 2015).

2.6.1 Post Intensive Care Syndrome

The rapid advancement in critical care medicine and subsequent increase in survival chances after severe illnesses have led the intensivists to identify an important functional disability that some surviving patients suffer (Hiser *et al*., 2023). Post Intensive Care Syndrome (PICS) is a disability acquired after surviving severe illness (Hiser *et al*., 2023). This is characterized by abnormal thinking, mental and physical disabilities of the ICU survivor (Needham *et al.,* 2012, Rawal *et al.,* 2017). However, when PICS involves the psychological health of relatives of the survivor post admission it is referred as PICS-family (PICS-F). Similarly, PICS involves ICU-acquired "neuromuscular weakness" (ICU-AW), disturbed thinking and judgment (Hiser *et al*., 2023). Abnormal psychological status arising after suffering from severe illness and persistent beyond discharge from the ICU (Rawal *et al*., 2017, Needham *et al*., 2012). Although PICS has been described as a public health menace, its main occurrence is still not reported (Hiser *et al*., 2023).

2.6.1.1 Mental impairment

Many studies have found an incidence rate of 25% of post ICU discharge survivors, with some studies reporting higher than these (Pandharipande *et al*., 2013, Davydow *et al*., 2013). The major predisposing factors associated with mental impairment in ICU patients are delirium (Lee *et al*., 2020). Glucose dysregulation, respiratory distress syndrome requiring ventilation, stroke, diabetes, hypoxia, and hypotension, severe sepsis, respiratory failure, chronic alcoholism, prior mental impairment (advance age, premorbid health condition) (Pandharipande *et al*., 2013).

2.6.1.2 Psychiatric illnesses

The probability of developing mental problem after ICU discharge range from 1-62% (Lee *et al*., 2020), some of these problems include anxiety, depression, and PTSD (Hopkins *et al*., 2005, Desai *et al*., 2011). Some risk factors of delirium include female gender, poverty, low educational status and use of painkillers and sedatives (Rawal *et al*., 2016, Jackson *et al*., 2014); however, these risk factors are also implicated in cognitive impairment (Jackson *et al.*, 204).

2.6.1.3 Physical

Common physical symptoms include ICU-AW which was reported to occur in up to 25% of ICU survivors (Chen *et al*., 2024). ICU-AW usually manifests in the form of quadri or tetraparesis, poor mobility, and recurrent loss of balance (Hermans *et al*., 2014, Fan *et al*., 2014). Many conditions were reported to be associated with ICU-AW such as chronic respiratory support (> 7 days), severe bacterial infection, organ failure and sedation (Chen *et al*., 2024).

Related to the saying, "prevention is better than cure" this applies to PICS as well, hence the most important preventive measure is to limit the use of deep sedation, encouraging early mobilization as well as physical and occupational therapy (Balas *et al*., 2014, Kress, 2013). These require a holistic approach for successful management and outcome (Ramnarain *et al*., 2021). Pre-admission history, medical history, mental and clinical status of patients, as well as history of adaptation to stress conditions may go a long way in preventing Post Intensive Care Syndrome (Ramnarain *et al*., 2021). The ABCDE first aid management in ICU has been used in the past with good preventive outcomes (Morandi *et al*., 2011, Pandharipande *et al*., 2010). This includes: **A**wakening (low sedation); **B**reathing (trial); **C**oordination of management and cooperation between various specialties; **D**elirium observation, assessment and treatment; **E**arly mobilization and ambulation in the ICU.

2.6.2 ICU stressors

The presence of unfamiliar people, frequent alarm beeps, sophisticated equipment, irritating odor and bright lights are some of the factors that contributed to the physical and psychological stress of patients admitted to the ICU (Novaes *et al*., 1997). Many studies have reported patients' experience during ICU admission (Soehren, 1995). De Meyer *et al* (1967) documents how various ICU equipment, noise, loss of time sensation and not being part of discussion going on around them tied them down (Tronstad *et al*., 2023). A study by Ballard *et al.,* 1981 of 22 patients identified the

presence of nasogastric tubes and movement restriction as the main stressor in the ICU (Ballard, 1981). A further study by Yarcheski and Knapp-Spooner, 1994 conducted on patients who undergo cardiac surgery, found that absence from home, pain, external discomfort and restricted lifestyle as the main stressors (Yarcheski and Knapp-Spooner, 1994). Stressors in the ICU are many; however, but classified into 4 type's being physical, environmental, intrapersonal and interpersonal stressors (Table 2.1).

2.7 DELIRIUM AND CRITICALLY ILL PATIENTS

Delirium is a neuropsychiatric disorder characterized by sudden onset of confusion and alterations of consciousness, resulting in increased complications, cost and prolonged hospital admission (Wu *et al*., 2023). An incidence rate of 29 to 64% was reported among medical in-patients and even higher in ICU patients (Shen *et al*., 2018). Delirium in the ICU setting is associated with adverse outcomes, being self-extubation, removal of indwelling catheters, prolonged ventilator dependence and lengthened ICU stays and prolonged mental deterioration (Skrobik *et al*., 2004). The work of McCusker *et al*., 2002 has shown that delirium is not associated with increased ICU mortality, but rather an independent marker for increased one-year mortality (McCusker *et al.*, 2002). Delirium may lead to altered memories, recurrent dreams and hallucination, all of which has been associated with PTSD symptoms in recent studies (Hatchett *et al*., 2010). In a study of ICU patients, 5% of patients were reported to not to be able to recall previous happenings in the ICU, 20% remembered a few events, and a further group of <10% of patients had less or total forgetfulness of the events, but only remember dreams, hallucinations (Roberts and Chaboyer, 2004). The research group concluded that, interval sedation is associated with good psychological and physiological results, compared to continuous sedation (Roberts and Chaboyer, 2004; (O'Connor *et al*., 2009).

Table 2.1 The various stressors in the ICU (Novaes *et al*., 1997).

Physical stressors	Environmental stressors	Intrapersonal stressors	Interpersonal stressors
Loss of muscle mass weakness	Alarm beeps	Poor or no patient autonomy	Difficulty in communication
Joint pain and stiffness	Sophisticated equipment	Constant need of support	Workload on staff
Poor sleep quality/ latency	Neighbouring patients	Loss of basic reflexes	Lack of human touch
Poor appetite	Phone calls	Talking to self	Family burden
Bedsores	Strange staffs	Delirium	Poor personality
Changes in skin turgor	24-hour artificial bright light	Fear of death	Bad manners among some staffs
Dyspnea on exertion	Poor privacy and restriction	Forgetfulness	Transfer stress
Haemodynamic in balance or postural hypotension	Persistent presence of staffs in unit	Sadness	Loneliness
Hair loss	Restraints	Financial burden	
Reduced power to expel mucus - pharyngeal weakness	*In situ c*annulas/ drains/monitors	Agitation	
	Discomforting procedures	Sober	

There are numerous predisposing factors responsible for acquiring delirium in the ICU which can precipitate and potentiate the delirium (Table 2.2). Symptoms of delirium during ICU admission have no feature relationship with the development of visual and perceived events. Many tools such as the Confusion Assessment Method for the ICU (CAM-ICU) and Depression Anxiety Scale (DASS) have recently been developed to screen and identify delirium in ICU patients (Fan *et al*., 2012). However, the management of these behaviors can be achieved by non-pharmacological measures. Antipsychotic administration is broadly accepted especially for agitated delirium (Choy *et al*., 2018). The use of antipsychotic agents is believed to shorten symptoms severity and duration; however, they are the most frequent risk factor for delirium in ICU (Ely *et al*., 2001, Skrobik *et al*., 2004). Administration of sedatives and painkillers like fentanyl, diazepam may cause prolonged periods of altered sensorium, forgetfulness and sedation (Franjic, 2023)

Table 2.2: Risk factors for delirium in ICU (Priscilla, 2019)

Benzodiazepines and narcotics	Creatinine ratio ≥ 18
Foleys catheter (bladder/ rectal)	AKI (creatinine > 2.0 mg/dl)
Impairment of sight and hearing	Congestive heart failure history
Central venous catheter	Malnutrition
High/low glucose level (<4.4 or 6.7 mmol/l)	Substance abuse
Increased/ decreased Na^+ level (<125 or >145 mg/dl)	Seizure, cerebrovascular accident history
Very low temperature (< 36°C or > 38°C)	Brought in from nursing home
Physical restraint	Hepatic disease (bilirubin >2 mg/dl)
Age > 70 years	Hypo/hyperthyroidism
Nasogastric tube feeding	HIV-infection
Previous depression history	Alcoholic
Shock due to microorganism/ cardiogenic shock	

2.8 OXIDATIVE STRESS IN BALANCE

2.8.1 Etiology of oxidative stress

Homeostatic conditions ensure a state of equilibrium between the amounts of free radicals formed and scavenging is maintained (Dresen *et al*., 2023). Excess free radicals are destructive, but their normal concentration is indispensable to the proper function of the body, with their production under a tight control by enzymatic and non-enzymatic systems (Sies *et al*., 2017). Cell growth, proliferation, apoptosis and differentiation are some of the vital processes free radicals partake in (Dabrowska and Wiczkowski, 2017). Oxidative stress is the process of disruption of cell homeostasis and shifts in the pro-oxidant/ antioxidant equilibrium in the direction of oxidation reaction (Sies *et al*., 2017). Critically ill patients suffer from an oxidative imbalance with resultant free radical elevation (Dresen *et al*., 2023). Alteration in both cortisol and free radical levels may result in worsening of the patients' condition (Dresen *et al*., 2023). The three main effects of oxidative stress include lipid peroxidation, protein degradation and DNA damage (Srivastava *et al*., 2017). This pro-oxidant/ antioxidant imbalance can cause serious damage to the biomolecular structures, such as protein, nucleic acid and lipid, but the former is the worst affected by the process (Mennear, 1998).

Development of atherosclerosis is characterized by aggregation of form cells which contain a large number of fat cells within the intima of the arterial wall (Dresen *et al*., 2023). However, this may lead to prolonged formation of free radicals (ROS) that has been linked to chronic inflammatory response (Chisolm and Steinberg, 2000). Oxidative process is an ongoing harmful chain reaction characterized by formation of Malondialdehyde (MDA) as the final marker (Vidyasagar *et al*., 2004) and bye product of total lipid peroxidation level and free radical damage resulting from oxidative stress (Tangvarasittichai, 2017). Studies have linked the psychological status of the patients to the lipid degradation product levels (Patterson *et al*., 1993). The main parameter of lipid profile in acute and chronic stress is hypercholesterolemia and has already been linked to the pathogenesis of angina pectoris (Agarwal *et al*., 1997). Acute stress situations have also been shown to reduce the fractions of red blood cells levels by causing the relocalization of white blood cells to defend areas of the body like lymph nodes (Qureshi *et al*., 2002). Many factors are associated with etiology of oxidative stress and its role in the pathogenesis of most diseases (Dresen *et al*., 2023).

2.8.2 Causes

The oxidative stress is referred to as inability of the reactive oxygen specie to be repaired by the body system (Kiran *et al*., 2023). Oxidative stress comprises a wide range of etiologies, from environmental, genetic, immune, and cellular factors and they are all interrelated to favor the oxidative stress in the pathogenesis of most autoimmune diseases (Kiran *et al*., 2023). Reactive oxygen species (ROS) are involved in a wide range of diseases, including chronic inflammation, a variety of cancers and various forms of autoimmune diseases (Table 2.3).

2.8.2.1 Environmental Causes

Oxidative stress may follow chronic exposure to environmental factors like ultraviolet radiation rays, toxins, and infective organism, lead poisoning that may ultimately cause serious damage to DNA, cell membrane and organelles (Zhou *et al*., 2023). Persistent environmental stress pollutants may lead to mutation in genetic make-up of organisms and subsequent damage (Semenkov *et al*., 2015). This ensures oxidative stress with resultant production of ROS by macrophages (Zhou *et al*., 2023). Titanium dioxide has been associated with disruption of mitochondrial activity in the liver cells probably due to strong oxidative stress induction (Natarajan *et al*., 2015). The organophosphate can cause indirect reactivation of Epstein-Barr virus (EBV) through ROS induction and DNA damage (Zhao *et al.*, 2015, Zepeda-Arce *et al*., 2017). Many pathogenic bacteria like *Mycobacterium avium,*

Escherichia coli, H. *Streptomyces* are implicated as the causative agent in some autoimmune disorders like Alzheimer's and many other diseases (Singh *et al*., 2015).

Table 2.3: Diseases caused by oxidative stress (Srivastava *et al*., 2017)

Autoimmune disease	Features	Etiology
Asthma	A respiratory problem characterized by obstructive airway disease	Hyaluronan structural defect and destruction of collagen 1 by radicals in pathogenesis of airway obstruction through tissue activation
Epilepsy	A degenerative episodic impairment of the central nervous system arising from the excessive discharge of a group of neurons	Associated with the role of Nrf2 gene which regulate antioxidant response elements
Atrial fibrillation	Characterized by abnormal heart beat, dyspnea, chest pain and abdominal pain	Inflammatory response and oxidative injury were showed by enzyme kinetics to significantly increased
Metabolic syndrome	Type 2 diabetes (insulin resistance)	Increased level of MDA, metabolism of carbohydrate, lipid and protein increased significantly
Parkinson's disease	Characterized by bradykinesia, muscle rigidity, resting tremor, posture and gait abnormalities	Progressive degeneration of dopaminergic neurons secondary to microglial activation, neuroinflammation, oxidative stress and apoptosis
Skin cancer	ROS altered apoptotic pathway	DNA damage and lipid peroxidation by oxygen radical species
Addison's disease	Adrenal hormone insufficiency	Symptoms include diarrhoea, abdominal pain, back pain, leg pain, hyperkalemia, low blood pressure
Schizophrenia	Mental disorder, abnormal thinking and behaviour	Genetic susceptibility, catecholamine metabolism

2.8.2.2 Genetic Causes

Cells are endowed with genes that respond to stress with the aim of improving stress. DNA mutation and damage may lead to modulation of innate-immune system with subsequent activation of mitogen-activated protein kinase enzymes whereby causing the transcription of pro- and anti-inflammatory gene and later activation of stress-response protective gene (Semenkov *et al*., 2015).

This ensures increased lipid peroxidation; generated metabolites help in generating more ROS thereby causing mitochondrial DNA damage (mtDNA) with resultant propagation of oxidative stress (Besaratinia *et al*., 2024).

2.8.2.3 Inflammatory Causes

Histocompatibility complex formation initiates a sequence of events that activate oxidative stress reactions, hence evoke autoimmune diseases. Generation of certain mediators such as cytokines and chemokines further mobilizes more inflammatory cells to the site of damage to generate more ROS. Induction of cyclo-oxygenase-2 and inducible nitric oxide (iNOS) induces the expression of inflammatory cytokines, TNF, interleukin-1 and chemokines, they are collectively responsible for the pathogenesis of various autoimmune disorders (Bhakat *et al*., 2009). The low level of heme-oxygenase-1, has been implicated in diseases like chronic inflammatory bowel disease (Crohn's disease and ulcerative colitis) (Singh *et al.,* 2004).

2.8.2.4 Cellular Causes

All aerobic organisms require oxygen for daily generation of energy through mitochondrial electron transport chains (Aramouni *et al*., 2023). Through this cellular process reduction of molecular oxygen occurs, generating ROS (Aramouni *et al*., 2023). These ROS systems activate many signaling pathways in aerobic cells, in response to intra and extracellular changes (Berlett and Stadtman, 1997). It has also been shown that mitochondrial reactive oxygen species have been implicated in aging related diseases (Nickel *et al*., 2015).

2.9 MELATONIN AND ITS PROPERTIES

2.9.1 Physiology of endogenous melatonin

Melatonin (N-acetyl-5-methoxytryptamine) was first discovered by Lerner A.B where he isolated it from bovine pineal gland (Lerner *et al*., 1958). It is said to be the most secretory hormone of the pineal gland in humans, however, other sites like testes, gastrointestinal tract and retina added small percentage of the hormone to serum level (Bubenik, 2002). Synthesized melatonin secretions commence 6-8 weeks after delivery then stabilizes at 3-5 years of age and progressively reduces through adulthood and old age (Cavallo and Ritschel, 1996). This indole compound has been linked

to various functions regulation among different species, including circadian rhythm, immune function, retinal function and most recently it functions as an antioxidant and free radical scavenger (Reiter *et al*., 2002, Tan *et al*., 2002). Melatonin demonstrated effectiveness in the treatment of sleep problems, jet-lag and sepsis, yet the role of melatonin in critically ill patients is ongoing (Bourne and Mills, 2006).

Melatonin release begins at night at around 21:00, maximum secretion was observed at about 22:00 and 4:00 respectively. Inhibition of these endogenous hormones begins around 19:00 and 21:00, coinciding with the peak level of endogenous cortisol. The blood concentration of endogenous melatonin is 0-20 pg/ ml in the day time but much higher at night reaching 40-100 pg/ ml (Cooper *et al*., 2000, Arendt, 2005). The 6-sulfatoxymelatonin (MT6) is considered as the main metabolite of melatonin, its blood level correlates with the urine MT6 level. The rhythmic secretion of endogenous melatonin is regulated by the sympathetic nervous system via β_1 and α_1 receptors (Arendt, 2005).

2.9.2 Melatonin as an Antioxidant/ Pro-oxidant

The human body possesses many natural antioxidants, such as melatonin to counteract an increase in free radicals (Ahmad *et al*., 2023). In vitro melatonin acts like glutathione and vitamin E in scavenging free radicals in order to reduce ROS (Arendt, 2005, Reppert and Weaver, 1995, Marshall *et al*., 1996). Supplementation with vitamins, trace nutrients and other antioxidants may help in restoring the redox equilibrium in critical illness (Ortiz Leyba *et al*., 2011). Melatonin is an indole amine with hypnotic, anti-oxidant, pro-oxidant and antiseptic action (Choy *et al*., 2018). Endogenous melatonin levels affect both night time peaks and basal diurnal levels in critically ill ICU patients, resulting in wake-sleep rhythm disorders (Claustrat and Leston, 2015; Mistraletti *et al*., 2017). It has been shown that melatonin levels are depleted under high oxidative stress conditions, but it remains unknown whether this is due to reduced endogenous production or increased metabolism (Mistraletti *et al*., 2017). Many drugs commonly used in the ICU (steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), benzodiazepines, β-blockers, α_2-adrenoceptors agonists, local anesthetics) inhibit melatonin production (Rahardjo, 2015). Melatonin and melatonin agonists have the potential to decrease the incidence and severity of delirium through their hypnotic and sedative-sparing effects, along with providing analgesic relief, thus improving health-related outcomes (Khella *et al*., 2018). Melatonin neutralizes lipid peroxidation, inhibits dopamine oxidation and reduces the risk of worsening degeneration and progression of Alzheimer's disease (Wu and Swaab,

2005, Acuna *et al*., 1997).

Anti-microbial activities of melatonin are one of its major pro-oxidant characteristics (Ben- Nathan *et al*., 1995). This factor is believed to arise from its ability to reduce bacterial lipid storage and iron binding capacity, causing bacterial substrate consumption (Tekbas *et al*., 2008). Melatonin has also been shown to reduce viral load as well as risk of pulmonary tuberculosis infection in animal models (Ben-Nathan *et al*., 1995, Valero *et al*., 2002).

2.9.3 Melatonin and Sleep

Melatonin plays a vital role in sleep regulation but the relationship between phases of sleep and melatonin concentration is not strong (Shilo *et al*., 1999, MacFarlane *et al.*, 1991). Patients with critical illness suffered from deprived sleep in the ICU. Melatonin level among these patients has been reported to be reduced significantly during their ICU stay (Weinhouse and Schwab, 2006, Shilo *et al*., 1999) in relation to day time basal level or nocturnal increase. Patients with an abundant release pattern, melatonin, induce sleep even during the daytime (peak level) (Middleton *et al*., 1997, Lewy and Newsome, 1983). Oral administration of melatonin may provide therapeutic relief to this group of patients, but the pharmacokinetics of the drug is not well understood (Mistraletti *et al*., 2010). The benefit of using melatonin includes increase in sleep efficiency, sleep time (TST), stage 2 and decrease in slow wave sleep in addition to sleep synchronization regulation (Patel *et al*., 2022). Some of the factors that have been reported as the possible cause of disturbed sleep among critically ill ICU patients includes drugs, psychological disturbances, frequent check-ups, noise, pain (Olofsson *et al.,* 2004, FRISK *et al*., 2004). Finally, it is not well understood if disturbance of sleep may arise from the ICU environment or reduce melatonin levels (Mistraletti *et al*., 2008).

The rate limiting enzyme responsible for the synthesis of melatonin is promoted by darkness with its activity regulated by many neurons in the suprachiasmatic nuclei (SCN) where circadian rhythm is programmed and modulated by peripheral stimuli (Brzezinski, 1997). Light and darkness act as the main triggers in balancing the pacemaker that regulates circadian rhythm. In humans, peak melatonin release coincided with the level of alertness and core temperature (Åkerstedt *et al*., 1979). It has also been reported that there exists a contradictory report on the relationship between melatonin rise and Electroencephalogram tracing during sleep despite sleep induction ability of melatonin (Swami, 2024). Melatonin has been implicated in seasonal variation in humans, thermoregulation and puberty as well as control of fetal circadian rhythm (Cagnacci *et al*., 1991,

Cagnacci *et al.*, 1997). Melatonin also stimulates the milk let down effect of prolactin from the pituitary gland (Waldhauser *et al*., 1987) with positive stimulation of LH pulsatile activity. At very high concentration melatonin lowers the reproductive function of mammals but improves fertility at lower dose that promotes circadian rhythm (Anderson *et al*., 1993).

2.9.4 Other Function of Melatonin

Melatonin has been demonstrated to reduce hormone dependent cancer progression probably due to circadian optimization cell function (Cagnacci *et al*., 1992). Melatonin can function in paracrine, autocrine and intracrine passion by down-regulation of iNOS (Hardeland *et al*., 2011). It also plays a major role in neuroprotection by inhibition of neurotoxic damage and preventing ischemia (Kilic, 2004).

2.10 SLEEP IN THE ICU SETTING

2.10.1 Normal sleep

Sleep function is indispensable for the maintenance of good health, and the need for sleep increases with illness (Frisk and Nordström, 2003). Sleep regulation is a complex process that involves various centres in the brain stem, hypothalamus, thalamus and forebrain (Patel *et al* 2022). The sleeping brain resembles an awakened brain in terms of activities as recorded using Polysomnography (Shirota *et al*., 2023). The ascending reticular activating system (RAS) plays a vital role in sleep- wake regulation and is mediated by GABAergic, adrenergic, cholinergic and histaminergic pathways. Both systems organize and conduct the normal sleep architecture, which is categorized into 2 phases, namely, non-rapid “eye movement (NREM) and rapid eye movement sleep” (REM) (Patel *et al*., 2008, American Academy of Sleep Medicine, 2018). Non-rapid “eye movement sleep is divided into 3 main stages” (stage 1, 2, 3 and 4). “Stage 1” is a transitional stage between wakefulness and sleep and at this stage the sleeper can be easily aroused. While stage 2 is a slightly deeper stage of sleep, where the sleeper is unaware of his surroundings, but is still easily aroused. “Stage 3 and 4 (delta or slow-wave sleep) is thought to be the most restful part of sleep, and during this stage an increase in growth hormone secretion and a decrease of body metabolism and cortisol secretion occur” (Patel *et al*., 2008). Rapid eye movement sleep is characterized by autonomic instability, active brain activity with absent muscle tone and is the stage where most dreaming is believed to take place

(Della Monica *et al*., 2018). A complete sleep cycle lasts for about 90 minutes, and on average people go through four to five cycles during a sleep period (Bourne and Mills, 2004).

Circadian rhythms refer to the internal biological clock that ensures self-regulation of sleep (Li *et al*., 2023). The clock is controlled by the suprachiasmatic nucleus (SCN) of the hypothalamus (Collop *et al*., 2008). Example of circadian rhythms in humans is the sleep-wake system (Li *et al*., 2023). The SCN regulates impulses which modulate or promote sleep including HPA and melatonin release from the pineal gland (Collop *et al*., 2008). The "time keepers" such as the light/ darkness cycle regulate the activities of this clock by oscillating from phase to phase. Circadian rhythm can be assessed through melatonin variation, cortisol and body temperature variation (Collop *et al*., 2008).

2.10.2 Disturbance of Sleep in Intensive Care Unit

Sleep is indispensable for the recovery of hospitalized patients, and sleep disturbance is an important outcome of critical illness (Altman *et al*., 2018). Sleep is a complex physiological process inherent to each individual and commonly covers nearly one-third of the life span (Mendonça *et al*., 2019). Hence critically ill patients admitted to the ICU need extra sleep (Mendonca *et al*., 2019). However, they are at higher risk for sleep deprivation, poor sleep quality and disturbed sleep-wake cycle (Altman *et al.*, 2018). Sleep allows daily repair of the major wears of various systems, including musculoskeletal, respiratory and CNS (Mendonca *et al*., 2019). Sleep also plays a role of consolidating memory, emotional regulation and improving quality of life (Mendonça *et al*., 2019). Sleep in ICU patients specifically, has been characterized by predominance of stage 1 and 2 sleep, poor or absent stage 3, 4 and REM sleep (Freedman *et al*., 1999). The work of Wilcox *et al*., 2018 reported that up to half of the critically ill patients have more sleep during the day time with NI and N2 NREM sleep (N1-stage 1 sleep, N2-stage 2 sleep) constituting a greater percentage of the total sleep time (Wilcox *et al.,* 2018). In the ICU setting sleep deprivation has been identified as a major problem, with more than 60% of ICU patients reported to suffer from sleep deprivation and sleep disorders during hospitalization (Rahimi *et al*., 2018). Orwelius *et al.* (2008) reported that 38% of the 1,625 study cohort found it difficult to fall asleep and 61% were in need of more sleep. Sleep deprivation is known to increase the risk of many of the clinical and physiological symptoms also found in delirium (Dessap *et al.*, 2015). Complications reported to be associated with sleep deprivation include abnormal respiratory mechanics, autonomic instability, impaired endocrine response, cellular and humoral suppression, psychological impairment such as poor intellectual performance, inattention and delirium (Bellapart and Boots, 2012). A sustained insufficient sleep

may predispose individuals to risk of metabolic disorders such as diabetes and cardiovascular pathologies like hypertension (Mendonça *et al*., 2019).

Critically ill patients have abnormal circadian rhythm control probably due to absence of timekeepers in the ICU as well as systemic inflammation (Rustam *et al*., 2023). A study reported by Haimovich et al., 2010 that injection of endotoxin in human participants dramatically changed the internal biological clock gene expression in white blood cells suggesting poor synchronicity between central and peripheral clock modulation of inflammatory response (Haimovich *et al*., 2010). Persistence day time urinary 6-sulfatoxymelatonin show marked circadian rhythm loss in septic patients, however, changes in circadian rhythm of melatonin secretion, reduces gene expression and elevated level of TNF-α and IL-6 as shown in sepsis patients (Mundigler *et al*., 2002, Li *et al*., 2013).

2.11 PHYSIOLOGICAL SEQUELAE OF SLEEP DISTURBANCE

2.11.1 Light-dark cycle

Limited light exposure has been shown to improve the incident delirium among ICU-patients through the process of sleep modulation (Collop *et al*., 2008). For instance nocturnal light exposure reduces the melatonin secretion from the pineal gland in response to darkness, resulting in recurrent wakefulness (Collop *et al*., 2008). Persistence light exposure in an animal model, a study by Gile *et al*., 2018, indicated reduction in gene expression of *per-2* in SCN with subsequent manifestation of delirium symptoms (Gile *et al*., 2018). However, a study among 195 critically ill ICU patients at University hospital, Paris, France reported that light exposure was associated with a reduced risk of agitation and hallucination (Smonig *et al*., 2019).

2.11.2 Noise in the ICU

WHO recommends 35 dBA of noise for nighttime in hospitals (Vreman *et al*., 2023), however, high levels have been reported in some ICU settings (mean, 53-59 dBA) with peak noise level of 67-86 dBA (Freedman *et al*., 2001, Freedman *et al.,* 1999). In a study by He *et al*., 2018 of noise level in a medical ICU shows noise levels in the ICU exceeded the recommended value, however, they recommended noise reduction strategies in ICU in the specific setting (He *et al.*, 2018). Many factors

contributes to the high sound levels in the ICU, including alarm beefs, mechanical ventilator machines and continues presence of staffs on duty (Gabor *et al.*, 2003). Studies using the gold standard sleep measurement tool (PSG) reported that 20% of ICU-patient arousals are due to environmental noise peaks and 35% of peaks greater than 75 dB (Cabello *et al*., 2008, Freedman *et al*., 2001). ICU noise levels have been shown to be similar during day and night time hours, contributing to poor or absence of REM sleep among the ICU-patient (Pisani *et al*., 2015). There are few studies that wanted to limit ICU-environmental noise, ear covers were recommended for the patients but not compulsory, hence inconsistency in the findings ensured (Bourne *et al*., 2008).

2.11.3 Sedation

EEG features of sedated patients are quite different from the natural sleep features (Jean *et al.*, 2023). Commonly prescribed sedatives among critically ill patients in the ICU include propofol and benzodiazepines, both GABA agonists with propofol being the first option (Fraser *et al*., 2013). Administration of sedatives like benzodiazepines results in poor sleep latency with dramatic enhancement of sleep architecture, poor SWS and REM stages (Plante *et al*., 2015). Propofol also causes poor SWS and suppression of EEG readings. Opioids analgesics commonly co-administered with sedatives in critically ill patients act via the ponto-thalamic pathways following binding to its μ-receptors; depending on the dose opioids analgesic can decrease slow wave sleep and REM sleep (Dimsdale *et al*., 2007). Recently introduced sedative agent, dexmedetomidine in the ICU has been shown to reduce the incident delirium without affecting the patient's quality of sleep reporting (Skrobik *et al*., 2018). Dexmedetomidine drug also enhances sleep efficiency and sleep time more than the GABA agonist. It's a α-2-adrenergic agonist, with anxiety reducing, sleep enhancing and pain relief action (Lu *et al*., 2017).

2.11.4 Patient Restraint

The use of physical restraint deprives patients of a day-to-day interaction with their environment (Huber *et al*., 2006). There are many conflicting reports concerning the use of restraint in the ICU, however, no single published report has yet proven the best form of restraints, whether chemical or physical that is in the patient's best interest, hence ICU staffs were advised to choose the correct, least restrictive method of restraint, while ensuring patients safety, with less guidance to support their decision-making (Freeman *et al*., 2016). Treating agitated delirium patients in the ICU whilst

preventing interference in treatment can be very difficult sometimes (Cohen *et al*., 2002). Such patients can harm themselves through displacement of intravenous-line and removal of artificial airways (Hine, 2007). In an extreme situation where risk of patients harming them is high, ICU staff may re-sedate the patients, which may negatively affect the patient's response to treatment and recovery (Mehta *et al*., 2011). In countries like Canada, the United States of America (USA) and the United Kingdom (UK), physical restraint is only recommended in prevention of the aforementioned behaviors (Freeman *et al*., 2016). It was suggested that physical restraint should only be aimed at reducing treatment interference while lowering the dose of sedation (Freeman *et al*., 2016).

2.11.5 Mechanical Ventilation

Recent study show strong evidence suggesting a link between patient-ventilator interaction and sleep disturbance directly or through need for higher doses of sedative drugs (Telias and Wilcox, 2019). Restlessness and wakefulness associated with breathing can interfere with patient-ventilator interaction and subsequent poor sleeping breathing synchrony which may normalize following removal of stimuli (Parthasarathy and Tobin, 2002). Central apneas were shown to cause sleep fragmentation including arousal and awakenings, similar to that experienced by patients with poor daytime sleepiness and impaired cognition (Tobin, 2018). Hence sleep deprivation in intensive care setting may be strongly connected with development of delirium, and consequently resulting in extended ICU stay, and increased mortality (Ely *et al*., 2004a). Disrupted sleep among mechanically ventilated critically ill patients can result from integrated malfunction of the CNS including impaired cognition and memory, immune system and body metabolism associated with poor prognosis (Jean *et al*., 2020). Other factors identified among human and animal models include, attenuated ventilation response to hypoxia and hypercapnea and increased oxygen consumption and carbon dioxide production respectively (Cooper *et al*., 2000). Sleep disruption, therefore, could interfere with the process of weaning from mechanical ventilation by its effects on pressure, regulation, and output of the ventilator pump (Cooper *et al*., 2000). Thus delirium in mechanically ventilated patients has been associated with up to 30% higher ICU, hospital cost and long term cognitive impairment (Milbrandt *et al*., 2004, Sakusic *et al*., 2018).

2.12 SLEEP MEASUREMENT TOOLS

Assessment of sleep disorders requires appropriate tools, and as such, many questionnaires have

been developed in the process to assess patients' quality of sleep (Amirifar *et al*., 2018). Questionnaires can be used as confirmatory reports on quality sleep or for the assessment of feedback response (Fabbri *et al*., 2021). There are basically two methods involved in quality sleep assessment (objective method versus subjective method). The most common objective method is Polysomnography (PSG), which is the most reliable and validated method available to measure sleep quality (Pisani *et al*., 2015). The limitation of PSG, as with other objective methods, is that it requires sophisticated equipment, and is time and resource consuming, as it requires expertise to report the results (Kamdar *et al*., 2012). In the subjective method type, the patients themselves will explain to the assessor their own perception of their sleep quality and quantity, usually with the aid of questionnaires (Fabbri *et al*., 2021). These methods require relatively little time to collect information on patients' sleep and to report the result (Fabbri *et al*., 2021).

"The Richard-Campbell sleep questionnaire (RCSQ)" is among the various tools that were developed by Richard Campbell et al., 2000 to assess quality sleep among critically ill patients in ICUs (Kamdar *et al*., 2012). It possesses unique features, such as its simplicity with short items, and visual state is used to measure sleep quality (Amirifar *et al*., 2018). Richard-Campbell *et al*. (2000) evaluated the reliability of the questionnaire through internal consistency and found a Cronbach's alpha score value of 0.9 (Murata *et al*., 2019). Since then it has widely been used in clinical studies for sleep quality assessment (Rahimi *et al*., 2018; Kamdar *et al*., 2012). Despite the ease of use and low cost of the RCSQ, concerns have been raised about the appropriateness of patients' self-reported sleep quality while they are sedated and/or delirious (Amirifar *et al*., 2018). Upon comparison of the perceptions of the patients and nurses, a poor agreement was reported, however the same study suggested that the RCSQ can be completed by the nurse on behalf of the patient (Kamdar *et al*., 2012). However, the generalization and differences between the patients are limited by the diversity of the patients studied and the use of differing and less-established statistical methods (Kamdar *et al*., 2012). As such, the objective of this sleep study will be to evaluate patient-nurse perception of sleep using the validated and more widely published RCSQ (Kamdar *et al*., 2012) in the general-ICU of an academic level 1 Gauteng hospital.

2.13 ICU-PATIENT ASSESSMENT

About 37 years ago (1987), many intensivists charged themselves with the task of scoring the severity of ICU patients with the intention of comparing them with the general population and

further evaluating the results (Mumtaz *et al*., 2023). The outcome of intensive care is dependent on many factors present on the first day in the ICU and on the patient's course of therapy (Le Gall, 2005). The ICU score is a number, where the higher the number, the higher the severity, while the probability concept is an attribute indicating the probability of hospital death of the patients.

Symptoms of anxiety and depression are important issues for general ICU survivors, as well as the significant relationship between post-ICU memories of intra-ICU anxiety and anxiety during recovery (Castillo *et al.,* 2016). Measures to reduce anxiety during critical illness need to be considered and evaluated for their long-term benefits for survivors of critical illness (Castillo *et al*., 2016). Many patients may survive critical illness, but the recovery process can be physically and emotionally challenging with many patients suffering adverse emotional outcomes (Castillo *et al.,* 2016).

Below is presented some of the assessment tools and categorical values used in assessing intensive care patients, along with those to evaluate delirium as recommended by the Society of Critical Care Medicine (SCCM) (Girard *et al*., 2008).

2.13.1. Simplified Acute Physiology Score

The new Simplified Acute Physiology Score (SAPS) version II provides an estimate of the risk of death without having to specify a primary diagnosis (Le Gall *et al.*, 1993) (Appendix A). SAPS II is comparable to Acute Physiology and Chronic Health Evaluation (APACHE II). Included are "17 variables, 12 physiological variables, age, type of admission (scheduled, unscheduled, surgical or medical) and 3 underlying disease variables (AIDS, metastatic cancer and hematological malignancy)".

2.13.2. Confusion Assessment Method for the ICU

The Confusion Assessment Method for the ICU (CAM-ICU), adapted from confusion assessment method and was designed for use in intubated patients and validated against a reference standard rater in mechanically ventilated ICU patients (Girard *et al.*, 2008). The CAM-ICU is the most commonly used tool for diagnosing delirium by intensivists and non- psychiatrists and was reported to have the best combination of ease, speed, reliability and validity (Girard *et al.*, 2008) (Appendix B). CAM-ICU provides a standardized rating of delirium, which was validated by expert opinion and Diagnostic and Statistical Manual of Mental Disorders Revised IV edition (DSM-IV-R) (Ely *et al*.,

2001). When using “CAM-ICU, delirium is diagnosed in two stages, the level of consciousness (level of arousal) is first assessed using a standardized sedation scale. One example, the Richmond Agitation Sedation Scale (RASS), is a 10 point scale ranging from +4 to -5, with a RASS score of 0 denoting a calm and alert patient (Sessler *et al*., 2002)”. By convention RASS score of -4 and -5 indicate coma. Depression Anxiety Scale. The Depression Anxiety Scale (DASS) shortened version has 21 items grouped into three categories, seven items per scale. The DASS scale was described by its developers as having strong estimated reliability when used in the general adult population and shows the convergent validity of the DASS to be superior when compared to other scales (Crawford and Henry, 2003). The DASS measurement is based on three dimensions, namely, depression, anxiety and stress (Appendix B).

2.13.3. Post-Traumatic Stress Disorder (PTSD)

Many mental disorders including post-traumatic stress disorders (PTSD) can arise from stressful events, including anxiety, depression and post-traumatic stress disorder. A study conducted by Scragg et al, to describe the incidence of PTSD in patients discharged from ICU found an incidence rate of 2.7% for women and 1.2% for men (Scragg *et al*., 2001). The PTSD (Appendix C) manifest as re-experience of the trauma and avoidance of stimuli likely to remind the patients of the trauma, these include memories and vivid images of the events that can be of such strong intensity that the person loses contact with the surroundings (Scragg *et al*., 2001). Another symptom is hyper-arousal, associated with difficulty with sleeping, concentration and irritability. Anxiety or depression may occur as a result of critical illness rather than the traumatic event itself (Scragg *et al*., 2001).

According to Scragg *et al.* (2001), the trauma symptoms checklist-33 (TSC-33) measures symptoms found in psychologically traumatized individuals (Scragg *et al*., 2001). Each TSC-33 item (including nightmares, feeling things are “unreal”, “feeling that you are not always in your body” and feeling tense all the time) is rated according to its frequency over the preceding two months, using a four-point scale ranging from 0 (never) to 3 (often). The construct criteria were taken from Diagnostic and Statistical Manual, 4th Edition (DSM-IV).

Generally antipsychotic and sedative medications are commonly used in ICU (Franjic, 2023). Therefore, organised assessment of sedation and agitation remains vital in estimating sedative medications and evaluation of agitated behaviour despite various scale limitations (Kenes *et al*., 2017). The consequences of insufficient sedation and analgesia being administered to critically ill

ICU patients can be quite significant, including self-removal of *in situ* catheters, intra-luminal tubes and restlessness, aggressive behaviors against care-givers (Franjic, 2023). Therapeutically, these drugs have inherent risks when used in excess or over a long period of time (Franjic, 2023). As such, the effect of haloperidol on the patients' stress hormones, sleep patterns and oxidative status warrants further investigation to evaluate the benefits or disadvantages of using haloperidol in ICU patients with acute stress.

2.14 NEEDS OF FAMILY DURING CRITICAL ILLNESS

Family members contribute to the ongoing involvement in patients' wellbeing (McKiernan and McCarthy, 2010). Patients also need their family members close to them the same way healthcare personnel need the family input during patient management (Nelson *et al*., 2006, Ekwall *et al*., 2008). Impaired cognitive state of the patients makes family members more relevant in decision-making on various treatment options in both acute and ongoing care (Szalados, 2007). Family members were shown to experience anxiety, depression and/ or PTSD (Aiken *et al*., 2010). In research conducted on ICU patient's families, found a positive correlation between the relative perception of patients' condition as critical, and their level of anxiety (Delva *et al*., 2002). Stress and anxiety of having critically ill ICU patients can affect the families, coping mechanism, adaptation, decision making and long-term chance of developing PTSD by family members (Azoulay *et al*., 2005, Yin King Lee and Lau, 2003).

2.14.1 ICU STAFF

There is a limited study report, on ICU intensivists staffing, including intensivists/ patient ratio despite their impact on patients outcome and cost of treatment (Afessa, 2006). Constant presence of nurses for patients in ICU enables them to perceive and identify warning signs and symptoms of psychological distress with rapid response to ease their distress (Bray *et al*., 2010). However, higher patient burden results in worse outcomes (Bray *et al*., 2010). Violent and confused patients were reported to be very difficult to manage, because it takes a long time for the staff to stabilize and calm them (Bray *et al*., 2010). Further heavy workload on ICU staff, such as patient load, prolonged ward rounds, skipping of routine activities due to higher number of patients were reported by a staff model that compares low intensivists/ patient ratio versus high intensivists/ patient ratio (Ward *et al*., 2013). Some studies found a significant relationship between nurse staffing and hospital acquired pneumonia, shock, myocardial infarction, sepsis, and length of hospital admission

(Amaravadi *et al*., 2000, Aiken *et al*., 2002). A systematic review study also provides evidence of a relationship between ICU nurse staffing and patient outcomes (Frith *et al*., 2010, Penoyer, 2010). The higher the number of ICU nurse staffing, the better the patient outcome (Frith *et al*., 2010, Penoyer, 2010). It has been reported that many South African ICU nurses and other relevant personnel are leaving South Africa at an alarming rate, probably due to poor financial and working environment, and hence only 26% of nurses staffing in ICUs are registered as critical care nurses (Scribante *et al*., 2004). Several studies reported a high prevalence of physician and nurse's burnout (Khan *et al*., 2023). This being due to, multiple shifts, long shifts, night duty, poor workplace organization, and work overload, were some of the risk factors reported (Ali *et al*., 2011, Ramirez *et al*., 1996, Kluger *et al*., 2003). These studies concluded that physician and nurse burnout is of significance for both quality of care and workforce stability, and should be documented in hospital staff monitoring models (Embriaco *et al*., 2007).

2.15 SOUTH AFRICAN STUDY

Studies similar to this research project undertaken are scarce in this part of the world, including South Africa. The researcher did not come across any study in South Africa whether qualitative or quantitative into this subject. However, many western countries have done similar studies (Peeters *et al*., 2017, Vermes *et al.,* 2001, Boonen *et al*., 2013) probably due to rising concern about stress in everyday life and poor outcome of critical illness. Moch *et al.,* (2003) conducted a study on pituitary adrenal hormone response in chronic stress, Hatchett *et al,* (2010) described psychological sequelae following treatment in intensive care unit, while Mathivha *et al.* (2002) reported an overview of critical care medicine in South Africa (Mathivha, 2002). Intensive care training was officially only introduced in South Africa in 1996, and done post-registration qualified as a nurse, and is available at both public and private sectors (De Beer *et al*., 2011). The structural model was predominantly based on those that exist in Europe, Australia and USA (Mathivha, 2002). Therefore, the research topic is unique, and will add relevance to health care provision geographically.

2.16 Theoretical Frame Work

2.16.1 Acute Care Model (Robert M. Palmer)

The Acute Care for adult patient's model of care was designed to reduce the incidence of functional disability of older adults occurring during hospitalization for acute medical illness (Palmer *et al*.,

1994). The Acute Care intervention was conducted on a medical–surgical unit with the objective of preventing loss of independence in the activities of daily living life (ADL) and to restore by the time of discharge ADL independence lost during the course of the acute illness and hospitalization. The Acute Care intervention used complementary principles of continuous quality improvement and comprehensive assessment in developing a new system of care for acutely ill adults (Landefeld *et al*., 1995). A multi-dimensional intervention included four key elements: a physical environment designed to promote patient functional independence and safety, patient–centered care delivered at the bedside by registered nurses in collaboration with interdisciplinary providers, comprehensive discharge planning undertaken early in the hospitalization and informed by the interdisciplinary team, and medical care review to assure quality of medication prescribing and clinical management.

2.16.2 Conceptual Basis

Acute illness is often a stressful experience for adults and their family caregivers. Already acutely ill and usually with multiple chronic conditions, elements of hospitalization, such as a hostile (unsafe and anxiety producing) physical environment and processes of patient care (e.g., restricting mobility), may contribute to the patient's loss of functional independence or a failure to restore physical functioning to baseline prior to the acute illness and hospitalization. In the conceptual model of dysfunctional syndrome, now referred to as hospital-associated disability (Covinsky *et al*., 2011), impaired homeostatic reserves and multiple chronic conditions predispose the older patient to functional decline. The emerging physical impairments that might affect cognition leading to delirium, anxiety and depressive symptoms. A further deleterious effect on patient functional status is the usual silo-based care that often excludes the patient or family caregivers from engagement in the patient's recovery from illness and return to independent functioning (Palmer *et al*., 1995). Hospital-associated conditions, such as falls with injury, incident delirium, ischemic pressure ulcers, and catheter-associated urinary tract infections, are common in patients over age 65 but are potentially preventable. The hostile environment, prolonged bed rest, under-nutrition/ starvation, immobility/ physical restraints, and depersonalization contribute to the risks of functional decline and culminate in a dysfunctional or disabled patient. However, any disruption to this functional decline could potentially improve hospital outcomes by mitigating the adverse effects of the hospital experience, such as by enabling patients to walk, initiate self-care, socialize with other patients and family members, obtain adequate sleep, and achieve adequate nutritional intake

[Palmer et al., 1998]. In particular, immobility has potentially devastating effects on the patient's functional status, and freedom to perform ADL such as transferring from bed to chair and walking. The acute care unit was created to systemically disrupt or reverse hospital-associated disability while making patient throughput more efficient and patient-centered

2.16.3 Development of the Acute Care Unit

The Acute Care unit intervention represents a major cultural change in the hospital care of adults. Focus of care is on the patient and not on single diseases, and the full intervention requires adaptation of the physical environment to enhance patient functional independence and safety, and a shift from multidisciplinary to interdisciplinary team-based care.

2.16.4 The Prepared Environment

Design of the first Acute Care Unit was informed by experiences in architectural design of acute-care and long-term care facilities, inpatient rehabilitation programs, and prior experience of clinicians and researchers (Palmer *et al*., 1994). To make the physical environment more home-like and minimize the risks of falls, confusion, anxiety, and deconditioning, the original unit was tiled, handrails were installed in hallway corridors, the hallways were uncluttered, and wallpaper and paint colors emphasized earth tones with contrasts between floor, wall, and ceiling to aid patients with impaired depth perception (Palmer *et al*., 2014). Diffuse lighting including wall sconces and wall lighting behind the patient's bed was added, door handle levers were installed, large clocks and calendars were added to help with orientation, bathrooms had elevated toilet seats, and wall hangings and carpeting helped to reduce ambient noise (Palmer *et al*., 1994, Landefeld *et al*., 1995). A greater focus on wellness, calming environments, privacy, and noise reduction is standard procedure in ICUs, reflecting hopes to enhance patient satisfaction and improve patient safety (Cahnman, 2017). Hospitals today must comply with the Americans with Disabilities Act (https://www.ada.gov/) with room specifications that are quite compatible with the prepared environment.

2.16.5 Patient-Centered Care

Patient-centered care is defined as "providing care that is respectful of, and responsive to, individual patient preferences, needs and values, and ensuring that patient values guide all clinical decisions" (Joseph *et al*., 2018). Patient-centered care considers patients' cultural traditions, their personal preferences and values, and the needs of their family members, and includes them as integral members of the interdisciplinary team when making clinical decisions. In Acute Care Units, healthcare providers with a vested interest in the care of older adults share responsibility with the attending clinician/ physician for management of the patient and interactions with other team members. In the model of interdisciplinary team-based care, health professionals coordinate care and communicate with one another as well as directly with the patient and family. Unlike traditional silo-based and multidisciplinary care, the team members in the ACE unit prioritize what professional services are needed and create greater efficiencies than are normally seen in usual care. Recommendations are based on agreed-upon standards of practice for patients on the Acute Care Unit.

The Acute Care Unit model benefits from the pivotal role of nurses who provide 24/7 bedside care, advanced practice nurses who typically oversee interdisciplinary rounds and connect with attending physicians and consultant daily. The protocols include preventative measures designed to prevent decline in the patient's performance, bathing, and dressing, transferring from bed to chair, toileting, and feeding. For patients who are already impaired in the daily activities, restorative guidelines are designed to help patients regain independent functioning and to inform further evaluation by physical and occupational therapists or care managers. The protocols weight heavily on patient mobility, ADL functioning, patient nutrition with specific goals, and attention to skin integrity, urinary and bowel continence, cognitive function (including maintaining/restoring normal wake and sleep cycles, and prevention of delirium), and augmenting hearing and vision (Palmer *et al*., 2014).

2.16.6 Pivotal Importance of Physical Functioning

The relationship of baseline functioning to the subsequent transition to post-acute care was validated in a secondary analysis of the first Acute Cure Unit intervention trial (Fortinsky *et al*., 1999). Particular attention was paid to cognition with a realization of the risk of functional decline

related to prevalent delirium (Inouye *et al*., 1993). Recognition of depression, although challenging with acutely ill and complex older patients, is considered important to the team and was shown to be associated with functional decline and increased risk of 3-year mortality (Covinsky *et al*., 1997, Covinsky *et al*., 1999). Severe medical illness and multiple chronic conditions often inform the interdisciplinary team of the patient's prognosis and appropriateness of transitioning from aggressive interventions to comfort care. The team-initiated family/ patient conferences are held when faced with uncertain clinical pathways, a transition to comfort measures, or review of the patient's goals of care. Subsequent analysis of Acute Care cohorts identified six independent predictors of 1-year mortality among survivors of hospitalization that included Acute Daily Life function and the predictive index has become a widely disseminated tool (Walter *et al*., 2001).

2.16.7 Planning for Transition to Home (Discharge Planning)

In Acute Care units, the process of planning for the patient's transition back home begins on the first day of admission. The assumption made by the interdisciplinary team is that if the patient was living at home, then the goal is to have them return home guided by the functional trajectory. The original Acute Care unit studies were not focused on post-acute transitions of care, but the team understood that patient clinical stability and physical functioning were keys to a safe transition. The patient-centric components of the Acute Care intervention are especially important in reducing the risks of post-acute placement or early unplanned hospital readmission (Naylor *et al*., 1999, Coleman *et al*., 2006). Importantly, acute care and transitional care interventions have been adopted by hospitalists in quality improvement programs.

2.16.8 Medical Care Review

In many Acute Care Units, the major role of advanced practice nurse is to educate and mentor interdisciplinary team members, the bedside nurses, support staff, and the attending physicians using techniques of academic detailing (Soumerai *et al*., 1990). Medical review includes oversight of quality measures and metrics and assuring adherence to contemporary practice. Medical care review also helps mediate conflicts in the direction of care that might arise among team members or the team and specialty providers. For example, through engagement with the patient and family, the interdisciplinary team may have recommended comfort care and a transition to hospice for a

patient with an advanced serious illness and frailty, but subspecialists may recommend invasive procedures in a desperate effort to cure the patient of a single disease. The leader of the team might interface with the subspecialty provider and advocate for the patient and family whose views differ from the specialist's. This advocacy role of the team leaders also applies when the patients are not receiving evidence-based treatments. For example, an attending physician might decline to prescribe oral anticoagulants to a patient with atrial fibrillation and risk factors for atheroembolic stroke because of a perceived greater risk of bleeding and injury from falls. Physician detailing could provide evidence-based arguments for anticoagulation, favoring benefits over risks for the individual patient. Through interdisciplinary team discussions, consistency in the views of team members is central when they interact with patients and families over any controversial or potentially divisive issues. Medical care review of medications in conjunction with clinicians and other team members is an important contribution of leadership in the Acute Care Unit. The researcher may refer to guidelines for the appropriate prescribing of potentially inappropriate medications or high-risk medications.

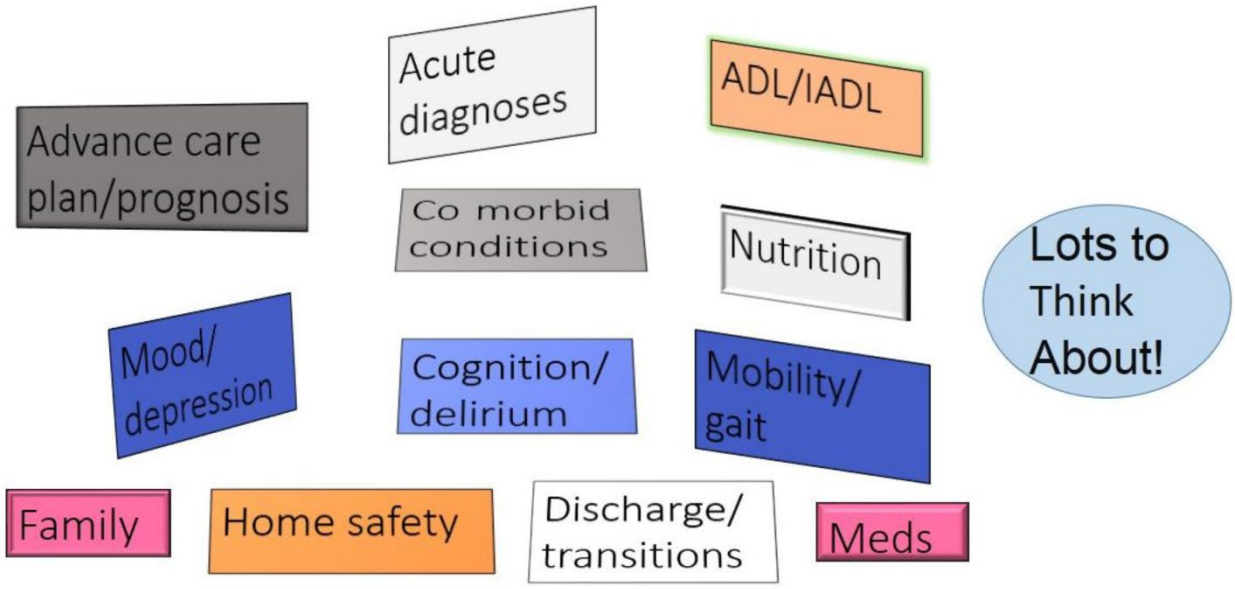


Figure 2.2: Complexity of hospitalized adult patients Acute Care Unit assessment (Robert *et al*., 2018).

CHAPTER TWO (2b) - HALOPERIDOL AND TRAMADOL MONOGRAPHS

2.1 INTRODUCTION

Haloperidol has been shown to be the drug of choice among patients with agitated delirium in ICU (Andersen-Ranberg *et al*., 2022). It was noticed during the pilot study that many patients in the study setting were on Tramadol as a primary painkiller used for the ICU patients, hence the need to include the drug in the study.

Haloperidol, a typical antipsychotic medication, is often used to treat hyperactive as well as hypoactive delirium in the Intensive Care Unit (ICU) (Girard *et al*., 2018). Haloperidol is lipophilic in nature with a molecular weight of 375.9 g/mol. Haloperidol acts by blockage of dopamine receptors in the brain and it is available in intravenous, intramuscular and oral preparations (Andersen-Ranberg *et al*., 2022). It is rapidly absorbed in the gastrointestinal tract following oral administration and undergoes first pass metabolism in the liver with only 1% of the ingested dose recovered unchanged in the urine (Khan *et al*., 2024). Tramadol is used to relieve moderate to moderately severe pain. It is similar to opioid analgesics with a molecular weight of 251.75 g/ mol. It acts in the brain to change how the body is perceived and how it responds to pain (Roldan *et al*., 2024). Tramadol is rapidly and almost completely absorbed after oral administration but its absolute bioavailability is only 65-70% due to first- pass metabolism (Lintz *et al*., 1998). Tramadol's metabolism is mediated by cytochrome enzymes and some of its metabolites are pharmacologically active, and hence contributes to the analgesic efficacy of tramadol (Budd and Langford., 1999). To determine the haloperidol and tramadol in plasma, the study makes use of a Flexar Perkin Elmer HPLC system, utilizing a RP column which is reported to have good selectivity and sensitivity, requiring simple sample preparation (Gu and Fawcett, 2005). The assay requires a small sample volume, involves a liquid-liquid extraction with a specific internal standard (sotalol) and a short chromatographic run (Jain *et al*., 2011).

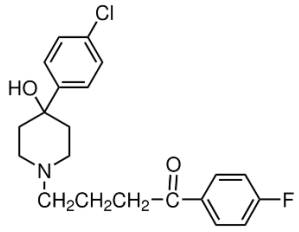
2.2 MONOGRAPH OF HALOPERIDOL

1. PROPERTIES OF HALOPERIDOL

Haloperidol is a crystalline material with very low solubility in water; however, it is soluble in

0.1 M hydrochloric acid with heating (Table 2b.1).

Table 2b.1: Properties of haloperidol (Drugbank, 2024).

Properties of haloperidol	Comments
Chemistry	
Physical properties	Haloperidol is a white to faintly yellowish amorphous or micro-crystalline powder
Melting point	Haloperidol melts in the range of 150 °C to 153 °C
Solubility	Haloperidol is freely soluble in Chloroform, Methanol, Acetone, Benzene and diluted acid. Haloperidol is practically insoluble in water
Dissociation constant	The dissociation constant (pKa) of haloperidol is reported as 8.6
Molecular structure	Molecular formula: $C_{21}H_{23}ClFNO_2$, Molecular weight: 375.9 g mol^{-1}

2. CLINICAL PHARMACOLOGY OF HALOPERIDOL

2.1 Preparation

Pharmaceutically haloperidol is available in different dosage forms, such as injectable and tablet (Table 2b.2). In medical slang, haloperidol is occasionally called vitamin H (O'Connor *et al*., 2019). It is sold under the trade names Aloperidin, Bioperidolo, Brotopon, Dozic, Droperidol (Germany), Haldol and Haloperidol (South Africa). In some countries, such as the United State of America, parenteral antipsychotics, such as haloperidol require a court order when requested by a psychiatric physician (O'Connor *et al*., 2019).

Haloperidol was first discovered by Paul Janssen in 1958 in Belgium, and approved by the U.S Food and Drug Administration (FDA) on April 12, 1967. Nearly 57 years after its discovery, haloperidol remains one of the most popular drugs used for the treatment of various classes of psychosis (O'Connor *et al*., 2019).

In comparison, atypical antipsychotics (Risperidone, Olanzapine, Clozapine and Quetiapine) have less extrapyramidal side-effects compared to typical antipsychotics like haloperidol (Abdullayeva and Nizamov, 2023). They are also effective against positive and negative symptoms of schizophrenia (Abdullayeva and Nizamov, 2023). Atypical antipsychotics also block both dopamine and serotonin (5-hydroxytryptamine) receptors (Abdullayeva and Nizamov, 2023). These combined actions on dopamine and serotonin receptors explain the lower extrapyramidal side effects of atypical antipsychotic drugs (Granger *et al*., 2005).

Table 2b.2 Haloperidol preparations (Drugbank, 2024a; Pubchem, 2024a).

Route of administration	Form	Strength
Oral	Oral concentrate (2 mg/mL)	0.5 mg 1 mg, 2 mg, 5 mg, 10 mg, 20 mg
Injectable solutions	Decanoate	50 mg/mL, 100 mg/mL
Per oral (PO) (Schizophrenia and psychosis)		0.5-2 mg, 8-12 hourly (moderate disease) 3-5 mg 8-12 hourly, not exceeding 30 mg/day (severe disease)
Intramuscular (IM)	Lactate(prompt acting)	2-5 mg 4-8 hours initially not to exceed20 mg/day
IM	Decanoate (depot)	IM dose: 10-20 times daily PO, reminder in 3- 7 days
Intravenous (IV)	Off-level	2-10 mg initially (monitor ECG and QT- interval)
Tourette disorder		0.5-2 mg PO 8-12 hours initially

3. MECHANISM OF ACTION

Haloperidol is a typical antipsychotic with all its pharmacological activity related to its affinity for the dopamine receptors (Chokhawala *et al*., 2020): Haloperidol blocks dopamine receptors in the mesocortex and limbic system leading to inhibitory action on dopaminergic and nigrostriatal pathways (Demirci *et al*., 2023).

Dopamine receptors are classified into five subtypes such as D_1, D_2, D_3, D_4 and D_5. These receptors are further divided into two subfamilies such as the D_1-like receptors (D_2, D_3 and D4) (Saeedi *et al*., 2006; Dearry *et al*., 1990). The binding of haloperidol to the postsynaptic D_2-like receptors is believed to mediate the therapeutic effects (Saeedi *et al*., 2006). Further, dopamine is a neuromodulator, acting in the brain by means of two important receptors (Demirci *et al*., 2023). The receptors being D_1 and

D_5 have similar chemical structures and intracellular signaling effects. The D_1-lke receptors have the ability to increase the levels of cyclic adenosine monophosphate (cAMP), while D_2, D_3 and D_4 receptors reduce cAMP levels and are termed 'D_2-like receptors (Taymans *et al*., 2003).

4. CLINICAL USES

The clinical uses of haloperidol in a study by Saeedi *et al*., 2006, of 59 ICU hospitalized patients (at University of Toronto) were those of delirium (69%), psychosis (11%), affective disorders/ dementia (11%), nausea and vomiting (9%) in an ICU setting. This study provides evidence that the major indication of haloperidol in clinical setting is for the management of delirium, and other psychiatric disorders (Saeedi *et al*., 2006).

Haloperidol is very efficient in treating the positive symptoms of schizophrenia (Ibragimov *et al*., 2024). The symptoms of schizophrenia include dystonia, tardive dyskinesia and Parkinsonian-like syndrome (Ibragimov *et al*., 2024). Haloperidol, despite being effective against positive symptoms of schizophrenia, is ineffective against the negative symptoms, such as lack of motivation, slow movement and change in sleep pattern (Glick *et al.*, 2001). Other indications for the use of haloperidol include neurological disorders such as Huntington's chorea and Gilles de la Tourette syndrome (Pubchem, 2024a).

5. HALOPERIDOL ADVERSE DRUG REACTIONS

A study by Valencia *et al*., 2023 showed that adverse drug reactions occur in ≥ 1% of haloperidol-treated patients and at higher rates than placebo in 3 double-blind, parallel, placebo-controlled, clinical trials with oral formulations, finding that nervous system disorders are commonest and includes hyperkinesia, tremor, hypertonia, dystonia, bradykinesia and somnolence, in that order (Karunarathna *et al*., 2023). However, less frequent gastrointestinal disorders include constipation, dry mouth and salivary hypersecretion respectively (Karunarathna *et al*., 2023). However, other adverse reactions that are reported by haloperidol-treated patients in double-blind, active comparator-controlled clinical trials with injectable or oral formulation include:

- *Cardiac Disorders*: Tachycardia
- *Eye Disorders*: Blurred vision

- *Endocrine Disorders*: Hyperprolactinemia
- *Investigations*: Weight gain
- *Musculoskeletal and Connective Tissue Disorders:* Muscle rigidity, Torticollis, Trismus and muscle twitching.
- *Nervous system Disorders:* Akathisia, Dizziness, Dyskinesia, Hypokinesia, Neuroleptic malignant Syndrome, Nystagmus, Parkinsonism, Sedation, Tardive dyskinesia
- *Psychiatric Disorders:* Restlessness and loss of libido
- *Reproductive System and Breast Disorders:* Amenorrhea, Galactorrhea, Dysmenorrhea, Erectile dysfunction, Menorrhagia, Breast discomfort
- *Skin and Subcutaneous Tissue Disorders:* Acneiform skin reaction
- *Vascular Disorders:* Hypotension and Orthostatic hypotension
- *Uncommon side-effect:* ECG changes (QTc-prolongation, torsades de pointes), photosensitivity reaction, generalized pruritus, blood dyscrasia
- *Rare:* Seizure, priapism and cholestatic jaundice (Pubchem, 2024a)

6. CONTRAINDICATIONS

Haloperidol is contraindicated if there is documented hypersensitivity to this drug, in Parkinson disease, dementia with Lewy body, comatose patient, in any condition with a depressed CNS (Karunarathna *et al*., 2023). Since many drugs such as barbiturates, benzodiazepines, and opioids can cause CNS depression, concurrent use of haloperidol should be avoided or used with great caution (Karunarathna *et al*., 2023).

7. DRUG-DRUG INTERACTION

Various drugs are known to interact with haloperidol (Roldan *et al*., 2024). Some of the known drug interactions for haloperidol are described and the information obtained from the following resources:[www.nhs.uk/.../5ml%20oral%20solution%20sugar%20free; psychrights.org/.../78-24-100324ExE13.pdf;www.namihelps.org/assets/PDFs/fact-sheets/Medications/Haldol.pdf.www.accessdata.fda.gov/.../015923s082,018701s057lbl.pd f]

- Combination of haloperidol and other CNS depressants such as sedatives, alcohol or narcotics, results in increased action and side-effects of these drugs such as sedation and respiratory

depression.

- The combined use of haloperidol and antihypertensive medications, such as methyldopa increases the risk of extrapyramidal side-effects (motor and CNS)
- Antiparkinson drugs like levodopa can be decreased when combined together with haloperidol.
- Haloperidol disrupts the metabolism and elimination of tricyclic antidepressant drugs. This will in turn increase the toxicity of these drugs, which include anticholinergic and cardiovascular effects and lowering of the seizure threshold.
- Other medications that are also metabolized by the CY3A4 enzymatic pathway can also decrease or increase plasma level of haloperidol. Quinine, buspirone and fluoxetine increase the plasma haloperidol levels while carbamazepine, phenobarbital and rifampicin decrease the plasma levels of haloperidol.
- The combined use of haloperidol and lithium has been shown to cause encephalopathy, extrapyramidal symptoms, coma and other neurological disorders.
- Haloperidol also reduces the plasma level of guanethidine, a blood pressure lowering drug.
- When haloperidol is used in combination with adrenaline it may lead to paradoxical reduction in blood pressure.

8. DRUG TOXICITY

Haloperidol toxicities are exaggerated symptoms of its known pharmacology and adverse reactions (Karunarathna *et al*., 2023). Typical symptoms include extrapyramidal symptoms, hypotension and sedation (Karunarathna *et al*., 2023). Patients may present unconscious, with severe respiratory distress and hypotension due to shock. The extrapyramidal symptoms present as muscular weakness, rigidity and localized or abnormal tremors (Karunarathna *et al*., 2023). Overdose from haloperidol may be life threatening with severe cardiac changes (ECG changes) known as torsade de pointes, which may result in abnormal heart rhythm and cardiac arrest (Wang *et al*., 2024).

Haloperidol toxicity has no specific antidote; management is mainly supportive (Brown et *al.*, 2024). Development of acute symptoms may require gastric lavage and or immediate induction of vomiting, followed by rapid administration of activated charcoal (Brown *et al*., 2024). The ABC emergency management protocol should be followed to ensure patent airway, good breathing and free circulation to ensure survival (Brown *et al*., 2024). A patent airway is secured with the use of

an endotracheal tube or tracheostomy, especially in unconscious patients. Artificial oxygen should be administered in case of severe respiratory difficulty or coma (Brown *et al*., 2024). In the case of hypotension, intravenous fluid, vasopressor agents and albumin should be administered (Rahman *et al*., 2020). ECG and vital signs are monitored at certain intervals. In case of extrapyramidal symptoms, antiparkinson medications should be considered. Monitoring of cardiac activity should be ensured until ECG normalization (Wang *et al*., 2024). If patients develop cardiac arrhythmias, antiarrhythmic drugs should be administered (Solmi *et al*., 2017).

9. PHARMACOKINETICS OF HALOPERIDOL

The pharmacokinetics and pharmacodynamics of haloperidol following administration by different routes are important considerations when it is used in delirium ICU patients, as the side-effects profile of haloperidol is significantly determined by its route of administration (Li *et al*., 2022). Several studies related to absorption, distribution, metabolism and excretion of haloperidol in animals and humans have been reported (Cressman *et al*., 1974; Cheng *et al*., 1992). In most of these studies pharmacokinetics parameters of haloperidol are usually reported after a single oral and intramuscular (IM) or intravenous (IV) administration in healthy volunteers and in schizophrenic patients (https://go.drugbank.com/drugs/DB00502). A summary of haloperidol pharmacokinetic parameters is shown in Table 2b.3.

Table 2b.3 Pharmacokinetic properties of haloperidol (Drugbank, 2024a; Pubchem, 2024a)

Half-life	12-35 hours (mean, 16 hours)
Oral bioavailability	44-75% (mean, 60%)
Volume of distribution	1280-2130 L
Steady state (time)	7-10 days
Protein binding	91.5 % bound
Measurable plasma concentration of haloperidol (time in hours)	1-1.5 hours (oral), immediate (intravenous), immediate (intramuscular).
Peak plasma concentration (time in hours/ minutes)	4-6 hours + 18 hours (oral), 5-15 min (intravenous), 20-40 minutes (intramuscular).
Decay post peak	Slow exponential (oral) steep over 1 hour then slow exponential (intravenous), like iv (intramuscular).

Haloperidol has a relatively complex metabolism and has fewer metabolites than other antipsychotic drugs (Skryabin *et al*., 2023). Haloperidol metabolism consists of glucuronidation to an inactive metabolite (50-60%), reduction and back oxidation to reduced haloperidol/ active metabolite (23%) and N-dealkylation to a pyridium metabolite which are toxic and constitute 20-30% (Pan *et al*., 1997).

Haloperidol has a relatively complex metabolism and has fewer metabolites than other antipsychotic drugs (Skryabin *et al*., 2023). Haloperidol metabolism consists of glucuronidation of inactive metabolite by 50-60% which then subsequently, back oxidized to reduced haloperidol which constitutes 3% (active form). The pyridium metabolite is the toxic metabolite constituting 20-30% (Pan *et al*., 1997).

In vitro studies reported by Wojcikowski *et al*., 2020 that cytochrome P450 2D6 enzymes (CYP2D6) is only partially involved in haloperidol's metabolism, however, *in vivo* studies suggested otherwise (Waaade *et al*., 2021). Haloperidol metabolism falls into two groups, namely slow and non-slow metabolizers. Non-slow metabolizers (Waaade *et al*., 2021), after receiving CYP2D6 inhibitors like paroxetine, can be rendered slow metabolizers Waaade *et al*., 2021 (). Slow metabolizers of CYP2D6 substrates have 30% higher haloperidol, 80% higher reduced-haloperidol plasma concentrations and 70% higher ratios of reduced- haloperidol to haloperidol than non-slow metabolizers. The CYPSD6 metabolizers show more effect on reduced-haloperidol concentrations than on concentrations of other metabolites suggesting that CYP2D6 is involved in the back oxidation of reduced-haloperidol to haloperidol. At a higher dose of haloperidol of 10-100 mg, no correlation was reported betweennCYPSD6 metabolizers probably due to cytochrome P450 3A4 (CYP 3A4) involvement in reduction/ back oxidation to reduced-haloperidol (>20 mg dose), but at a lower concentration this correlation was positive (McGrane *et al*., 2022).

Reduced haloperidol is not available in the plasma following oral administration for many hours and the regression ratio of the two shows no correlation pattern (Cheng *et al*., 1992). Detection of intravenous haloperidol takes a longer time than that of oral administration, suggesting strong first-fast metabolism, as the reason for increased reduced- haloperidol concentration (Figure 2b.1).

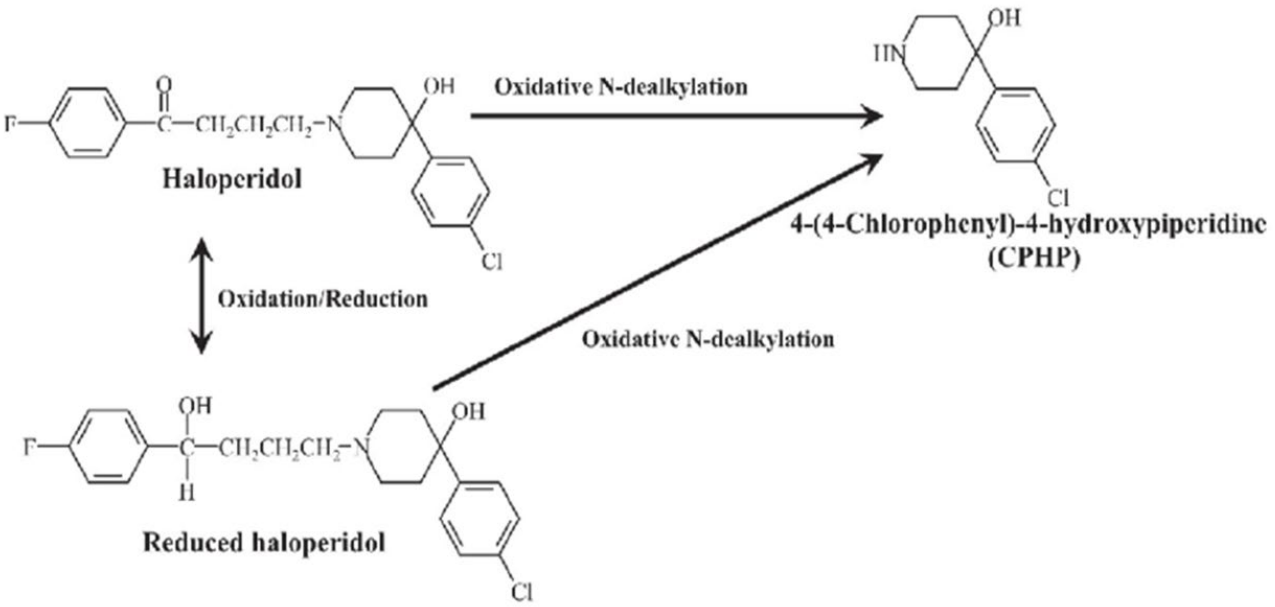


Figure 2b.1: Metabolism of haloperidol and reduced-haloperidol (Park and Shin, 2016).

2.3 MONOGRAPH OF TRAMADOL

1. PROPERTIES OF HALOPERIDOL

Tramadol is a synthetic codeine analogue that has central analgesic properties, with effects similar to opioids, such as morphine and codeine. Tramadol act on specific opioid receptors, namely, μ-opioid, k-and ɗ-opioid receptors (Table 2b.4).

2. CHEMISTRY

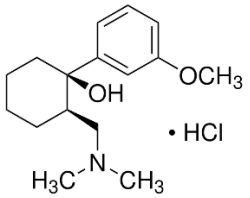
Tramadol was first developed in 1962 in Germany where it was first approved for human use in treatment of moderate to severe pain for the first time (Grond *et al*., 2004). Tramadol is a commonly used analgesic in ICU for moderate to severe pain (Siddiqui *et al*., 2023). It has 2 chiral centres and is a 1:1 racemic mixture of 2 diastereomeric enantiomers, which have different potencies with respect to monoamine reuptake inhibitors (Gong *et al*., 2014).

3. PHARMACOLOGY OF TRAMADOL

Tramadol is a centrally acting pain reliever acting on μ-opioid receptors (agonist) and is also a serotonin/norepinephrine reuptake inhibitor (SNRI) that is structurally related to codeine and

morphine (Siddiqui *et al*., 2023). Tramadol has a weak attachment to k- and δ-opioid receptors and to the μ- opioid receptor with over 5000-fold less affinity than morphine (Barakat *et al*., 2019).

Table 2b.4: Properties of tramadol (pubchem.ncbi.nlm.gov/compound/Tramadol).

Properties of haloperidol	Comments
Chemistry	
Melting point	Tramadol melts in the range of 180 to 181 °C
Solubility	Tramadol is soluble in water and methanol
Dissociation constant	Tramadol has a dissociation constant of 9.41. The log partition coefficient (logP) in n-octanol-water is 1.35 at pH 7
Molecular structure	Molecular formula: $C_{16}H_{25}NO_2$, molecular weight: 263.40 g/ mol

Tramadol exists as a racemic mixture with two pharmacologically active enantiomers that both add to its analgesic property through different mechanisms (Siddiqui *et al*., 2023). The isomer (+)-O-desmethyl-tramadol inhibits norepinephrine reuptake (Grond *et al*., 2004). These pathways are complementary and synergistic in improving tramadol's ability to modulate the perception and response to pain (Grond *et al*., 2004).

3.1 Dose and formulation

Tramadol is available in an oral formulation, intravenous solution, and rectal suppository (Ishitsubo *et al*., 2024). Immediate-release and delayed-release formulations are available as well as combined forms with other drugs such as paracetamol (Ishitsubo *et al*., 2024). The normal dose of tramadol ranges from 50, 75, 100, 150, 200, 300 and 400 mg. At first it is taken at 25 mg per day, the dose can be increase as needed and tolerated, however, the dose is usually not more than 400 mg per day (Miotto *et al*., 2017).

4. PHARMACOKINETICS

After administration, both the [-] and [+] iso forms of tramadol, and the M1 metabolites are detected in circulation (Grond *et al*., 2004). Following administration, racemic tramadol is rapidly and almost completely absorbed with bioavailability of 75% (Table 2b.5). The difference in absorption and bioavailability can be attributed to the 20-30% first-pass metabolism (Grond *et al*., 2004). The peak plasma concentration of tramadol and its main metabolite M1 occur at 2 and 3 hours, respectively. After a single oral dose of 100 mg of tramadol, the C_{max} is found to be 300 µg L^{-1} with a T_{max} of 1.6 to 1.9 hours while M1 was found to have a C_{max} of 55 µg L^{-1} with a T_{max} of 3 hours (Grond *et al*., 2004).

Table 2b.5: Pharmacokinetics of Tramadol. (Pubchem, 2024b)

Items	Properties	Comments
Volume of distribution	Volume of distribution: 2.6-2.9 L/kg after oral administration Volume of distribution: 306L and 203L after parenteral administration	Category C Drug
Protein binding	Significant percentage of the drug is protein bound (20%)	Protein binding appears to be independent of concentration up to 100 µg/mL Saturation only occurs at concentration outside of the clinical range
Half-life	Tramadol half-life, 5-6 hours M1 metabolite, 8 hours.	Half-life is longer with metabolite
Clearance	Clearance of tramadol ranged from 3.73 ml/min/kg in renal impairment to 8.5 ml/min/kg in healthy adults	Reduce dose in renal diseases
Elimination	Primarily metabolized in the liver Metabolites primarily eliminated by the kidneys (90%), remaining 10% in the faeces	Approximately 30% of the doses is excreted in the urine unchanged whereas 60% excreted as Metabolite.

4.1 Metabolism

Tramadol undergoes extensive first-pass metabolism (Table 2.5) in the liver by N-and-O-demethylation and conjugation from the extensive metabolism (Dean and Kane, 2021). About 23

metabolites were formed during metabolism (Dean and Kane, 2021). However, only two of this metabolite (M1 and M2) have clinical relevance, and are both catalysed by CYP3A4 and CYP2B6 (Figure 2b.2).

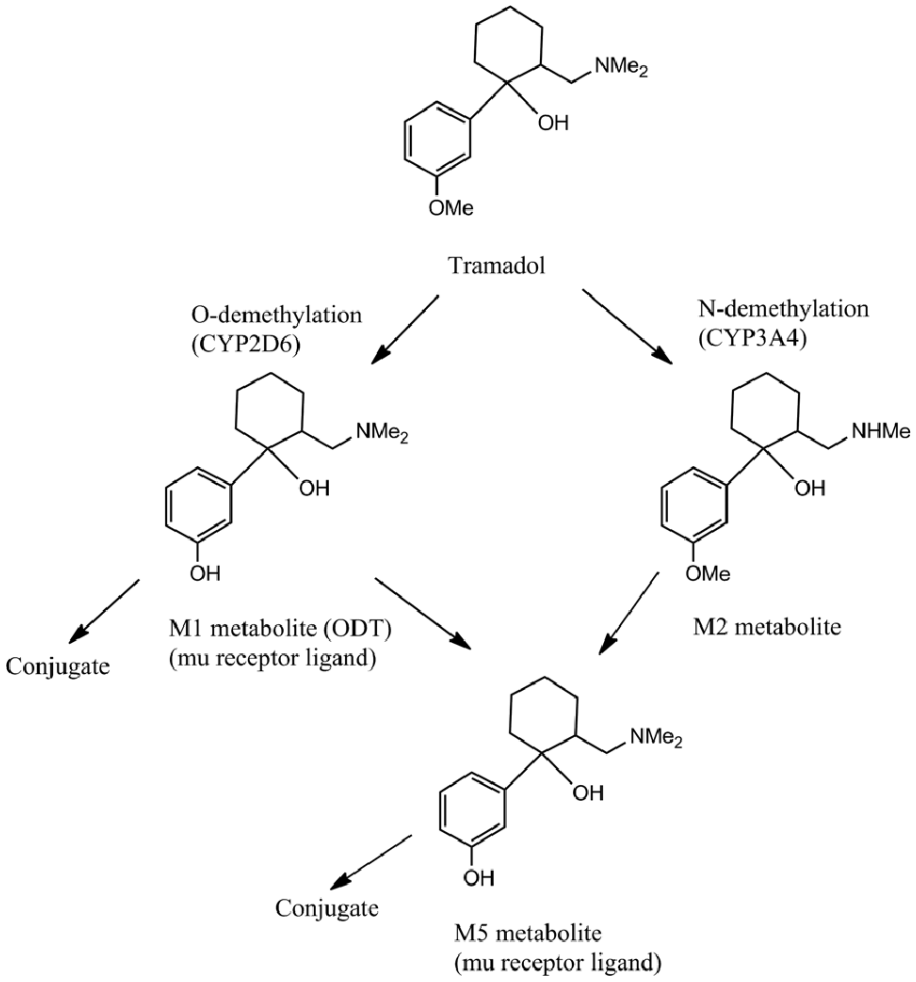


Figure 2b.2: Phase 1 metabolites of tramadol (Miotto *et al*., 2017).

5. PHARMACODYNAMICS

Tramadol shows symptoms that are similar to other opioids relievers such as dizziness, nausea, constipation, sweating and pruritus (Mahmoud *et al*., 2022).

- **Cardio Vascular System effect of Tramadol**: In contrast to morphine, tramadol has no histamine release effect (Mahmoud *et al*., 2022). Tramadol has no effect on heart rate or left ventricular function, but patients have been reported to have observed hypotension (http://www.accessdata.fda.gov/drugsatfda_docs/lebel/2010/022370s000lbl.pdf). Severe hypotension may result from patients with compromised blood pressure or concurrent

administration with antidepressants, tranquilizers and sedative/hypnotics. Special care should be exercised when administering tramadol to patients who are at risk of experiencing torsade pointes during treatment with QTc-prolongation drugs (http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022370s000lbl.pdf).

- **Respiratory effect of Tramadol:** Tramadol produces respiratory depression by direct action on the brain stem respiratory center (Mahmoud *et al*., 2022). Tramadol also acts on the cough center to depress the cough reflex. It can cause miosis, even in total darkness (Mahmoud *et al*., 2022).

- **Central Nervous System effect of Tramadol:** Seizure can occur even at the recommended dose of tramadol. It also increases the risk of convulsion in patients with epilepsy and should be avoided in the at-risk population (Auro Pharma Inc., 2018). A rare life-threatening condition may result when tramadol is administered together with serotonergic drugs such as antidepressant and migraine medications. The condition is characterized by hyperthermia, rigidity, clonus, and autonomic instability with fluctuation in vital signs, changes in mental status, including confusion, irritability, agitation, delirium and coma. Tramadol should be avoided when MAO inhibitors are used to avoid serotonin syndrome (http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022370s000lbl.pdf).

- **Gastro Intestinal Track effect of Tramadol:** Tramadol causes slow motility with increase in smooth muscle tone in the duodenum and antrum of the stomach. Tramadol also delays food digestion in the small intestine secondary to propulsive peristalsis waves (http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022370s000lbl.pdf).

- **Endocrine System Effect of Tramadol**: Opioids like tramadol may influence the hypothalamic-pituitary-adrenal or gonadal axes by decreasing testosterone production, and also affect the level of luteinizing hormone (LH), Follicular stimulating hormone (FSH) and prolactin (http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022370s000lbl.pdf).

6. ABUSE AND MISUSE

Like all opioids, tramadol has the potential for abuse and misuse, which can lead to overdose and death. Therefore tramadol should be used with caution (Abd-Elkader *et al*., 2020). Tramadol is thought to be a drug of abuse because of its weak opioids activity, but no well-established

documentation of the evidence exists (Miotto *et al.,* 2017). However, physical dependence has been reported with chronic use of tramadol (Lanier *et al*., 2010). Therefore, with the increasing popularity of tramadol, physicians must be aware of its adverse effects, substantial abuse potential, and drug interactions, to weigh its risk–benefit ratio for pain management (Nakhaee *et al*., 2021). According to the WHO report tramadol abuse is prevalent in West Africa, North America, Middle East and Asia (http://who.int/medicines/areas/quality_safety/6_1_update.pdf). There are few studies in humans reported on tramadol dependence (Shalaby *et al*., 2022). Death has been reported in association with tramadol use, this has doubled in some countries by ten percent especially in Northern Ireland for the last sixteen years (Randall *et al*., 2014).

CHAPTER THREE

RESEARCH DESIGN AND RESEARCH METHODOLOGY

3.1 INTRODUCTION

This chapter discusses the research methodology that was applied in this study. The research design was prospective in nature with a quantitative approach. The qualitative methods were used to describe standard management of critically ill patients in the ICU of university teaching hospital being a tertiary level institution, in region of Johannesburg, Gauteng province, South Africa. This chapter also provides an insight into the method and procedures employed to accomplish the aim and objectives of the study.

3.2 STUDY DESIGN

The study used a prospective mixed method design. Quantitative data were collected from subjects in each of the delirium and non-delirium patients every day through the period of data collection. Sleep quality were measured using RCSQ as well as the factors that promote or deter sleep. The researcher administered the RCSQ, CAM-ICU at least ones. Qualitative data collected through brief interview between researcher and patients were aim at understanding how patient sleep and what factors deter his sleep. Data collected at multiple points during the patient's admission (on admission to ICU, during and on discharge from ICU) to investigate select variables used (patient assessment tools, laboratory investigations) and can be assessed (Appendix D). Other necessary information was collated from patient ICU records. After consultation with the University of the Witwatersrand statistician, the sample size of 100 was determined to be required for statistical significance, where 50 patients who meet the criteria for haloperidol will be selected and 50 patients who will not receive haloperidol due to exclusion criteria. The instruments to be used at multiple points to assess the select variables, including Richard-Campbell Sleep Questionnaire (Appendix E), Depression Anxiety Stress Scale (DASS) (Appendix B), "Simplified Acute Physiological Score (ASPS II) and Post-Traumatic Stress checklist, as well as Confusion Assessment Method for the ICU patients (CAM-ICU)" (Appendix B).

T**able 3.1**: Summary of general ICU setting

S/N	Item	Number/ activity	Comment
1	Nurses Staff	Twelve nurses at a time Nurse: patient ratio = 1:1	Nurses staff runs a shift duty at least three times a day (morning, afternoon and night)
2	Bed capacity	General ICU has twelve bed capacity	Two open ward with open space in between that serves as nursing station
3	Number of delirium patients	Twenty delirium patients Identified using CAM-ICU	These patients were also on antipsychotic, haloperidol
4	Role of researcher	Morning, afternoon and evening	The researcher is responsible for taking samples, administering questionnaire to the patients and reviewing of patients files (including patients folder and nurses documentation)

3.3 STUDY SITE

Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) is an academic level 1 hospital serving as a major tertiary referral center in Johannesburg and surrounding communities. It has a total of 1190 beds, and the ICU patients comprise approximately 6% of the total patient admission (Hatchett, 2010). About 12 patients are admitted at once in the general medical ICU, with some patients spending a longer time on admission (Personal communication, Associate Professor Schmollgruber, CMAH ICU nurse). The patients who utilize the hospital facilities are usually those that have no private medical aid cover and many foreign patients also use the facility. The nurse to patient ratio at this hospital in the ICU is 1:1 (Hatchett, 2010). A record review conducted by Scribante *et al*., in 2006 over a three month period found 306 patients and the mortality rate of 28% (Scribante *et al*., 2004).

3.4 STUDY POPULATION

The inclusion criteria for this study included all adult patients ≥18 years treated in general ICU for more than 24 hours, and patients, who understand and respond to the English language, hear and see. The exclusion criteria included, (i) patients who have predisposing risk factors for HPA-axis dysfunction, for example, conditions known to disrupt the hypothalamic-pituitary-adrenal axis such as the use of glucocorticoids and pre-existing hypocortisolism, patients with liver cirrhosis, pulmonary embolism and myocardial infarction. (ii) History of pre-existing sleep disorder, excessive

consumption of alcohol or substance abuse, suspicion or diagnosis of dementia or admission to ICU was due to violence and lastly, (iii) patients who do not wish to be included in the study. Patients receiving haloperidol were compared to those not requiring the drug. Plasma samples from routine blood measurements, which are drawn twice a day by ICU nurses, were used by the researcher to run various tests in the laboratory. Urine was drawn from the urine bags. All plasma and urine samples were stored until required at -70°C freezers in one of the division of pharmacology laboratory. The blood and urine samples were processed and prepared before storage.

3.5 VALIDITY

A pilot study was conducted and validity scores done to ensure feasibility of the study and to detect possible limitations in the instrument used (patient assessment tool and drug utilization documents). Content validity was achieved by having the data collection instrument reviewed by expert specialists (professor of critical care medicine and a professor of nursing education/ intensivists in the ICU). A prospective study takes notes of outcomes during the study period and relates this to other factors such as risk or protection factors. The outcome of interest should be common; with avoidance of sources of bias such as the loss of individuals to follow up during the study. Prospective studies usually have fewer potential sources of bias and confounding (Allen *et al*., 2024).

3.6 RELIABILITY

Reliability is maintained by ensuring consistency and accurate recording of data. Data collection was solely done by the researcher using amongst others the ICU patient forms that are of a high standard in recording patient vitals. Similar to previous reliability studies (Izah *et al*., 2023), the sample size of 100 patients was calculated by using the formula $n= Z^2 pq/d^2$ (n= desired sample size, Z= standard normal deviate, p = proportion in the target population estimated to have a particular characteristic, q = 1-p (proportion in the target population not having the particular characteristics, d= degree of accuracy required) assuming a two sided; 5% margin of error and power of 80% was used (Izah *et al*., 2023). This was determined to be representative of the study population, and taking into consideration possible patient not participating and sample degradation rates. Sample inclusion and exclusion criteria were also adhered to.

3.7 DATA COLLECTION

3.7.1 Profile of critically ill patients

All the patients used in this study were critically ill. The patient's clinical diagnosis and management details were obtained from the patient folder and used to describe their profile. Only patients admitted into the General ICU of University Teaching Hospital were included. Originally, a total of 100 (n=100) participants were selected for inclusion into the study. The reason for this number was based on consultation with a University of the Witwatersrand biomedical statistician. However, six participants declined permission for their data to be included in the sleep component of study, hence their data were removed from the main data used for final analysis, leaving a sample size of 94 (n=94).

3.7.2 Procedure

The study was described as prospective in nature with a qualitative approach. A structured record review collected the data over a period of time from the ICU charts of patients who had been admitted from day zero until discharge, or deceased.

3.7.2.1 Descriptive

Description, as a design, allows researchers to observe and report happenings as it occurs (Teodoro *et al*., 2018). It also describes how patients perceive happenings around them as well as other activities happening to them in the General ICU of a tertiary teaching hospital. The study also involved collection of data pertaining to the clinical profile of the respective critically ill patients admitted to the ICUs by means of a prospective record review conducted in the same patients.

3.7.2.2 Qualitative

Descriptive, as a design, as its main objective, is the desire to find out an explanation for the existence of a phenomenon, how it occurs and using a range of quantitative methods to collect data that aid in accurately describing a research problem (Bloomfield *et al*., 2019). Description comes from the data and study context. In this study, a descriptive design was employed to describe the characteristics of critically ill ICU patients and nurses response with regards to patient's perception in a General ICU of a tertiary teaching hospital in Johannesburg, South Africa.

3.7.2.3 Data collection method and tools

A record review was employed and data collected over a period of time from the ICU charts/ records of patients who had been admitted from day one till discharged or diseased.

Data collection included recordings for severity of illness on admission as well as records used by nurses to describe their perception on the level of improvement of each patient while on admission. Some of the variables measured using the tools include age, gender, SAPS II score, laboratory investigations (E/U/Cr, LFT, AST etc), quality of sleep assessment length of stay (calculated based on admission and discharge dates) and outcomes (discharged or died). The items contained in the measurement scale were well studied in various literatures prior to the commencement of the study (Kamdar *et al*., 2012, Scragg *et al*., 2001).

Each collection tools were completed for one patient's at a time while on admission by the researcher before proceeding to the next one.

- **SAPS II score:** This measured once in 24 hours and consists of 12 items (each item was scored between 0 to 26 points), ranging from age of the patients to cardiovascular parameters, body temperature, patients oxygenation as well as various laboratory test results. These test results were obtained from the patient's folder and ICU charts. A total SAPS II score was calculated by adding all the scores of selected items and scored appropriately to reflect the level of severity of illness of ICU patients. SAPS II validity was previously established by Le Gall (1993), it was reported to be 0.88 and 0.9 respectively. However, in South Africa only two recent studies were found to validate this important severity scoring instrument (Kisorio *et al*., 2009; Schmollgruber, 2015).

- **Depression Anxiety and Stress Score:** This is measured once in 24 hours and scored 0, 1, 2 and 3, this indicates how much the statement applied to the patients over a particular period of time; however, the patient's response to the questions does not permit a right or wrong answer. This score was obtained after the patient was discharged to the ward.

- **Post-Traumatic Stress Disorder:** This is measured within weeks or months after discharge. In this study it was measured within a month after patients were discharged to their respective wards. The tool examined the patient's experience after discharge and or during follow-up in order to assess patients' experience after ICU discharge. The seven questions range from patients' experience, flashbacks, dreams, anxiousness and avoidance of activities or persons

that reminded them of their previous experiences.

3.7.3 Study Division

3.7.3.1 Part 1 of the study

The study was divided for easy understanding into three parts. The part 1 of the study comprised the collection of data for better understanding of standards of care for each respective patient's on admission, and which consisted of three steps.

- **Step 1**- All information relating to the patients were collected using ICU records and patients ICU charts (record review), this allows for proper profiling of the patients.
- **Step 2**- In this step all nurses' documentation and side notes regarding patient care were reviewed and documented, for instance nurses' perceptions on patients' sleep. This allows proper understanding of how nurses perceive care of their patients.
- **Step 3**- Information regarding things that enhanced or retarded patients' sleep were asked directly from the patients in order to make sense of their own perception and to be able to compare them with the nurses' perception.

3.7.3.2 Part 2 of the study

This comprises mainly the laboratory based studies, and involves using blood samples obtained from the ICU patients in part 1 of the study. This part consists of two steps:

- **Step 1**- Involved preparation of stored blood samples for the laboratory assay
- **Step 2**- HPLC analysis was performed to determine haloperidol and tramadol in delirium patients.

3.7.3.3 Part 3 of the study

For this part of the study, cortisol and melatonin levels were determined by ELISA kits using the patient's plasma (Figure 3.1**)**. However, the concentrations of the haloperidol and tramadol were determined using a spectrophotometer. This part was also a laboratory-based study. It also involved 2 steps:

- **Step 1**- Treatment of the EDTA-drawn blood with antibodies
- **Step 2**- Measurement of the amount of light absorbed by each drug (based on calculation).

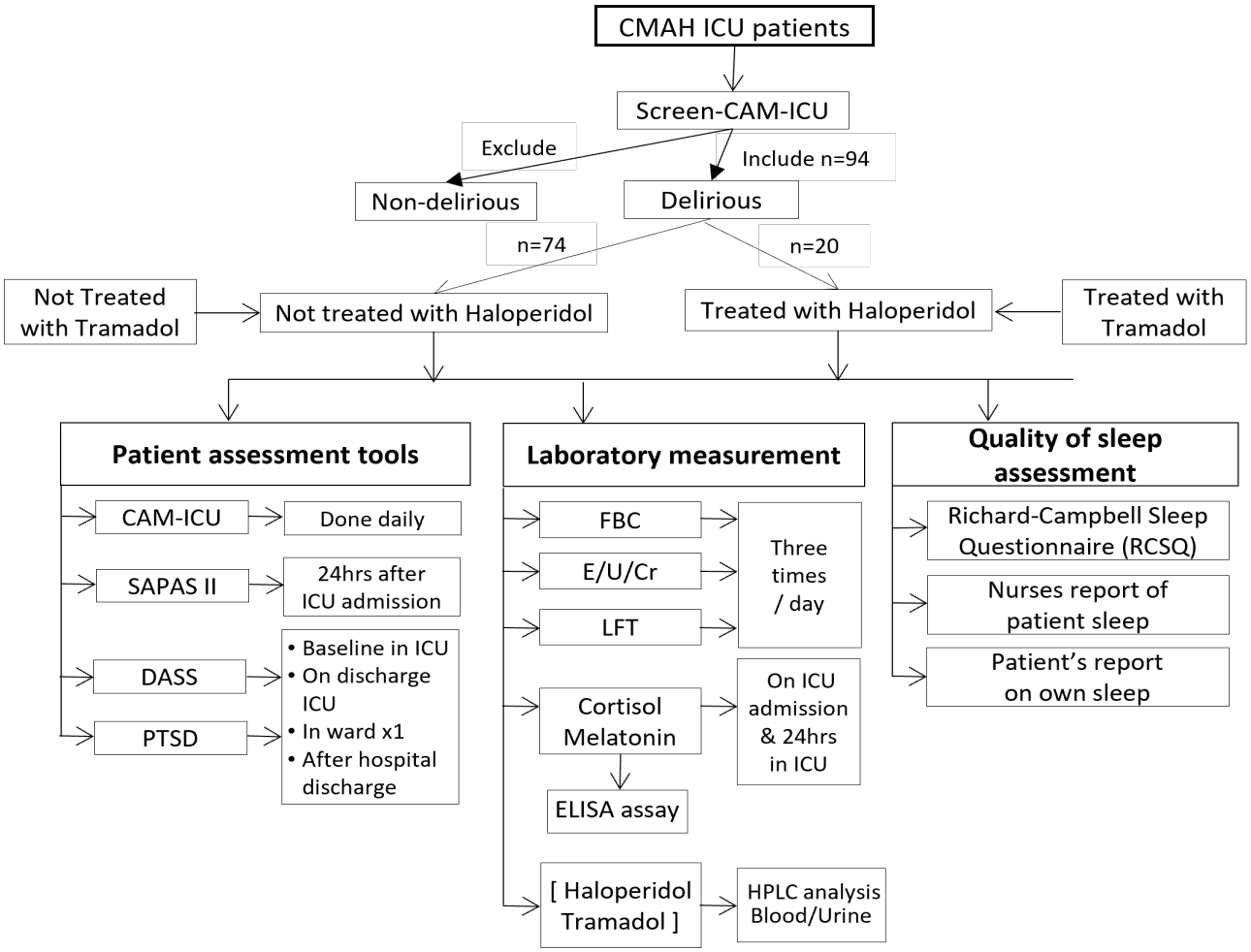


Figure 3.1 Overview of research design and methodology.

3.8 SLEEP ASSESSMENT

A series of assessment was obtained from each of the participating ICU patients as follows:

3.8.1 Patients Assessment of Sleep

The RCSQ consists of five items pertaining to sleep (sleep depth, falling asleep, awakenings, returning to sleep and quality of sleep) (Appendix E). The responses were recorded with a higher score representing better sleep and the mean score of these 5 items known as the "total score". The researcher with the assistance of the nurse on duty completed the questionnaires *in lieu* of the patient, but where the patient was found to be stable; the questionnaire was answered by the patient. A sixth item was added to RCSQ as in previous studies to evaluate perceived nighttime noise (range: 0 mm for "very quiet" to 100 mm "very noisy") (Chen *et al*., 2024). Personal demographic patient information pertaining to age, sex and race was obtained from the patient's medical record and reported on the data collection sheet specifically developed for the study (Appendix E). The

patients' medication list was used to obtain information regarding amongst others sedatives and hypnotics given to the patient. Ward patients report were used to determine on activities or interventions that promoted or deter sleep, while on admission in the ICU. "What things woke you up or kept you awake during the previous night?" These questions were communicated to the patients verbally, writing or through the patient action by the researcher (Appendix F).

3.8.2 Nursing Assessment of Sleep

The nurse's documentation of the patient's sleep was reviewed and recorded. If not documented it was requested to be reported as follows; "no sleep" (awake all night), "minimal sleep" (2-3 hours), "moderate sleep" (4-6 hours), "majority sleep" (7-12 hours) or "sleep all night" (≥ 12 hours). The nurses' documentation of patients sleep data was done retrospectively by the researcher.

3.9 BIOCHEMICAL AND HAEMATOLOGICAL VARIABLES

The variables that were evaluated included the following that was collected from the ICU sheets (Appendix D; G): Electrolyte, urea and creatinine (E/U/Cr), including aldosterone; Full blood count (FBC) and Liver function test (LFT). Pituitary-adrenal-hormones included cortisol, GH (growth hormone), prolactin and Adrenocorticotropic hormone (ACTH). Catecholamines investigated include adrenaline and noradrenaline. The investigations were routinely requested by a team of clinicians during daily ward rounds. Other investigations included are insulin, glucose, cholesterol triglycerides and free fatty acids. However, not all investigations were available for use by the researcher. The body mass index (BMI) was calculated from the patient weight and height (weight over height square). Quantification of dehydroepiandrosterone-sulphate (DHEA-S) was not frequently requested for each patient except where there was particular interest for those patients. Total cortisol was determined from plasma samples and urine cortisol was evaluated using an ELISA kit. The albumin levels were determined in order to calculate the free cortisol levels present.

3.10 MATERIALS AND METHODS FOR HPLC

3.10.1 Reagents and chemicals

All standards and HPLC/analytical grade laboratory chemicals were purchased from Sigma-Aldrich Co LLC (St Louis, Mo, USA) or Merck Co. LLC (NJ, USA). These standards included the following; haloperidol, tramadol, and sotalol was used as internal standard (Table 3.1).

3.10.2 Method Development For The Estimation Of Haloperidol And Tramadol By RP-HPLC

3.10.2.1 Wavelength selection

Two standards of 100 µg/ mL and 1000 µg/mL of haloperidol and tramadol respectively were prepared in methanol. The wavelength at which the compounds gave their maximum absorbance was investigated. Initially the wavelength of 254 nm was used then to 250 nm and then optimized at 240 nm in the UV-Visible spectrophotometer for haloperidol. The same was repeated for tramadol, and the wavelength of 250 nm was used for detection.

3.10.2.2 Chromatographic conditions

In the process of method selection for a particular analytical procedure, many factors are considered (Siddique *et al*., 2023). For example, ionic nature of the sample or its neutrality, the molecular weight and solubility (Siddique *et al*., 2023). In this study the selected drugs are lipophilic in nature (haloperidol and tramadol) hence the reverse phase HPLC was selected for the separation because of its simplicity, ruggedness, broad application and suitability (Siddique *et al*., 2023). The method was adopted from previous studies (Siddique *et al*., 2023).

3.10.2.2.1 Initial separation conditions

The mobile phase employed to elute both haloperidol and tramadol from the stationary phase was acetonitrile and phosphate buffer, making it suitable for UV transmittance, and having low retention on analytical column and low back pressure.

3.10.2.2.2 Effect of buffer

The mobile phase pH was optimized using different pH levels. The pH was initially adjusted to 3.64 and later lowered to 2.8 (pH adjusted with few drops ortho-phosphoric acid), at a flow rate of 1 mL min^{-1} and utilizing a Phenomenex Kinetex column (RP-C18: 5µ, 150 mm x 4.6 mm). The peaks and resolutions were monitored at different pH levels (3.64 and 2.8) and optimized to 2.8.

3.10.2.2.3 Ionic strength measure

The phosphate buffer was prepared in one single concentration strength, of 0.05 M of potassium monophosphate at pH of 2.8. Potassium monophosphate (6.8 g, KH_2PO) was weighed into a 1000 ml beaker and dissolved and diluted with 800 ml milli Q water. The flask was shaken and stirred until the particles dissolved, after which 1 ml of acetic acid was added. The pH was adjusted to 2.8 with

ortho- phosphoric acid and 200 ml of milli Q water was added to make up 1000 ml. The buffer was filtered through a 0.45 µm filter.

The retention time was decreased by raising the buffer strength. After optimization, the mobile phase composition consisted of: phosphate buffer at pH of 2.8: acetonitrile (50:50 v/v). The retention times of haloperidol and tramadol, respectively were sensitive to changes in ionic strength during analysis (Jain *et al*., 2011).

Analyses were performed using the following instrument and RP column system.

a. Perkin-Elmer Flexar HPLC system; components included: a binary pump, an autosampler, a column oven, an UV detector and Perkin-Elmer Chromera® software.

b. A Phenomenex Kinetex column (RP-C18: 5 µ, 150 mm x 4.6 mm) as well as C18 Security guard ultra-cartridge (4.6 mm)

The preliminary investigation showed that the column utilized, provided satisfactory results with the respective peaks (Figure 5.1, 5,2, 5.3 and 5.4) being with sharp definition, and good resolution, and hence it utilized for further analysis undertaken.

3.10.2.3 Extraction of volunteer/blank plasma and patient plasma

Extraction of haloperidol from human plasma samples was done via liquid-liquid extraction techniques (Jain *et al*., 2011). Human plasma samples (300 µL) were mixed with 50 µL internal standard (sotalol, final concentration: 87.5 ng/mL of 700 ng/mL stock solution) as well as 50 µL blank plasma or stock standard solution and vortexed for 60 seconds. Then 300 µL of 2-propanol was added and centrifuged at 9000 rpm for 5 minutes. The clear organic layer was separated and evaporated in a TurboVap LV Evaporator (Zymark, Hopkinton, MA, USA) at 50°C under a stream of nitrogen. The dried residue was then reconstituted with 300 µL of mobile phase and 20µL was injected for HPLC analysis (Jain *et al*., 2011).

Patient plasma was treated similar to the volunteer plasma above, with the addition of internal standard (sotalol), treatment with 2-propanol, drying, reconstitution in 300 µL mobile phase and injection of 20 µL samples into the HPLC system (Jain *et al*., 2011).

3.10.2.4 HPLC detection of haloperidol and tramadol

3.10.2.4.1 Method development and optimization

The initial method was development based on the chemical properties of the drugs and methods as developed by Kazusaki *et al*. (2012). Following various optimization steps the final method to estimate haloperidol and tramadol in the patient's plasma by RP-HPLC was eventually achieved by using the following chromatographic conditions (Table 3.1).

Table 3.1: Method development and optimization of haloperidol and tramadol by RP-HPLC

Chromatographic conditions:	Initial method development	Optimized method
Mobile phase	Methanol: Acetonitrile (50:50 v/v)	Methanol: Acetonitrile (50:50 v/v)
Flow rate	1 mL.min^{-1}	1 mL.min^{-1}
Column	C18 (4.6 x 150mm, 5µ)	C18 (4.6 x 150mm, 5µ)
Detector wavelength	240 nm	240 nm – haloperidol 250 nm - tramadol
Column oven	Ambient	Ambient
Injection volume	20 µL	20 µL
Run time	2 minutes	2 minutes – haloperidol 3 minutes - tramadol

3.10.2.4.2 Haloperidol and tramadol stock preparation and chromatograms

The mobile phase was prepared by mixing 100 ml KH_2PO_4 buffer and 100 ml of acetonitrile HPLC grade in a 50%:50% ratio. The mobile phase was used as diluent in preparation of stocks in mobile phase. Preparation sample stock solutions:

Haloperidol and tramadol (5 g) were accurately weighed (separately) and transferred into two 100 ml clean dry volumetric flasks. Diluent (5 ml) was added to each flask and the solutions were stirred so that the compounds dissolved completely, after which the solutions were then filtered through 0.45 µm syringe filters. These stock solutions were used for further dilutions (stock 1 solution). From the original stocks, a further 1000 ng/mL of both haloperidol and tramadol were made to at least 5 ml and these stocks (stocks 2 solutions) were used for further dilutions for the respective standard curves. Stocks were also prepared using volunteer (blank) plasma and compounds extracted as described in Section 3.10.2.4 above.

Standard samples and; blank preparations (20 µl) in triplicate were injected separately into the HPLC system and the peak responses for haloperidol and tramadol were measured. The developed RP-HPLC method for the estimation of haloperidol and tramadol was carried out on a Phenomenex Kinetex RP C18, 150 x 4.6 mm, 5 µm column in isocratic mode using a mobile phase composition (50:50 v/v) consisting of 0.05 M phosphate buffer (pH 2.8) : acetonitrile with flow rate of 1 mL min^{-1} at 240 and 250 nm, respectively. The individual chromatograms of haloperidol and tramadol standard samples, chromatograms for optimized methods are shown on Figure 5.2 and 5.4 respectively (Chapter five, Section 5.0).

3.10.3 Method Validation

The objective of the validation of the analytical procedure is to show its suitability for its intended purpose (Marson *et al*., 2020). As enshrined in the ICH guidelines, typical analytical performance characteristics that should be considered in the validation of the types of methods are: specificity, linearity, accuracy, precision, and limit of detection, limit of quantification, robustness and stability (Kazusaki *et al*., 2012).

3.10.3.1 Specificity

It is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include impurities, degradants and matrices (Vinay *et al.*, 2012).

3.10.3.2 Haloperidol and Tramadol identification

Separate solutions of standards in mobile phase and volunteer/ blank plasma were prepared according to the test procedures and injected into the HPLC system. Acceptance criteria for all the chromatograms of the standards in mobile phase and volunteer/blank plasma were considered to be identical with same or near the same retention time (acceptance criteria <2.0%)

3.10.3.3 Blank interference test

A study to examine the interference of the blank was conducted. Mobile phase or volunteer /blank plasma was injected into the HPLC system according to the test procedure.

Acceptance criteria

- The chromatograms of the blank should not show any peak at the retention time of any analyte peak.
- If there is no interference due to the blank at the retention time of the analyte, it indicates the specificity of the method (Van Iterson., 2011).

3.10.3.4 Linearity

Linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of the analyte in the sample (Vinay *et al*., 2012).

3.10.3.4.1 Preparation of stock solutions

Haloperidol and tramadol working standards were accurately prepared as stated in section 3.9.8.1 above. The solutions were then vortexed thoroughly to solubilize the haloperidol and tramadol after which the stock solutions were filtered through 0.45 µm syringe filters and stored at 4°C. For the plasma extraction refer to section 3.9.6 above.

3.10.3.4.2 Preparation of working solutions and chromatograms

A total volume of 5.0 ml of each working solution for both mobile phase and plasma was prepared from the stock solution (1-200 ng/mL) by using the equation $C_1V_1 = C_2V_2$. Each working standard solution (20 µl) was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was constructed and the correlation coefficient was calculated. The linearity of the method was demonstrated over the concentration range of 1-200 ng/ ml for haloperidol and tramadol respectively. The samples were injected into the HPLC system as per test procedure (Jain *et al*., 2011).

For the volunteer plasma standards, the compounds were extracted from the plasma according to section 3.9.6 above and 20 µl was injected into the HPLC system.

3.10.3.4.3 Acceptance criteria

- As far the test procedure (adapted Jain *et al*., 2011) the correlation coefficient (r) should be not less than 0.9990.
- The percentage (%) Relative standard deviation (RSD) of the peak areas for each concentration should be not more than 2% (Haem, 1992)

3.10.3.5 Accuracy

Accuracy expresses the closeness of the agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness (Vinay *et al*., 2012). Analyses were performed in triplicate for three concentration levels of haloperidol and tramadol, equivalent to 3, 150 and 200 ng ml^{-1} of the standard concentrations which were injected into the HPLC system as per the test procedure.

3.10.3.5.1 Preparation of Standard Stock and Working Solutions

Both haloperidol and tramadol stock and working standard concentrations were prepared as in section 3.10.2.2. From each of the above freshly prepared working solutions, 20 µl were injected into the HPLC system. The amounts calculated from these fresh chromatograms and amounts expected as per the standard curve for haloperidol and tramadol were compared and the mean recovery for each was calculated.

3.10.3.5.2 Acceptance criteria

The mean percentage (%) recovery of haloperidol at each spike concentration should be not less than 98% and not more than 102% (Hearn, G.M., 1992).

3.10.3.6 Precision

Precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions (Heam, 1992).

3.10.3.6.1 . Repeatability

Standard stock and working solutions

Both haloperidol and tramadol stock and working standard concentrations were prepared as in sections 3.10.2.1 and 3.10.2.2. The working standard solutions were injected six times into the HPLC system and the areas were measured for all six injections in the HPLC system. The % RSD for the areas of all six injections was then calculated.

Acceptance criteria

- All individual assays for haloperidol should be within a range of 98 - 102%
- %RSD of assay results should not be more than 2.0% (quansysbio.com/support/recovery-and-linearity/#).

3.10.3.6.2 Intermediate precision (Ruggedness)

Intermediate precision or ruggedness of the method is evaluated on different days using the same column and under the same conditions.

Standard stock and working solutions

Both haloperidol and tramadol stock and working standard concentrations were prepared as in Sections 3.10.2.1 and 3.10.2.2. The working standard solutions were injected six times and the areas for all six injections were measured as mentioned above.

3.10.3.6.3 Limit of Detection (LOD)

LOD of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantified as an exact value (Vinay *et al*., 2012).

Working standards of 20 ng/ ml for both haloperidol and tramadol were prepared as in Section 3.10.2.1 and 3.10.2.2.From this working stock, a 0.025ng/ ml solution was prepared by pipetting 1 ml of the working stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent. A further 1 ml of the latter stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent. 0.25 ml of the 1 ng/ ml solution was pipetted into the 10 ml of volumetric flask and diluted up to the mark with diluent. The standards were then injected into the HPLC machine to obtain chromatographs.

The LOD is determined by the formula: LOD= S/N; Where S= signal obtained from LOD solution (0.25% of target assay concentration)

Acceptance criteria: S/N Ratio value must be not more than 3 for LOD solution (https://www.google.com/search?q=R.A.+van+Iterson+Drenthe+College+Emmen+Holland+for+www.standardbase.com&oq=R.A.+van+Iterson+Drenthe+College+Emmen+Holland+for+ www.standardbase.com&aqs=chrome..69i57.2481j0j15&sourceid=chrome&ie=UTF-8).

3.10.3.6.4 Limit of Quantification (LOQ)

LOQ of an individual procedure is the lowest concentration of analyte in a sample which can be quantitatively determined with a suitable level of precision and accuracy (Vinay *et al*., 2012).

Working standards of 10 ng/ ml for both haloperidol and tramadol were prepared as in Sections 3.10.2.1 and 3.10.2.2. From this working stock, a 0.1 ng/ ml solution was prepared by pipetting 1ml

of the above stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent. 1 ml of solution was pipette into a 10 ml of volumetric flask and diluted up to the mark with diluent. The standards were then injected into the HPLC machine to obtain chromatographs. The LOQ was determined by the following formula LOQ= S/N; where S= signal obtained from LOQ solution (1% target concentration); N= Average Baseline Noise obtained from Blank solution; where the acceptance criteria for the S/N Ratio value was considered to be 10 for LOQ solution.

3.10.3.7 Robustness

The robustness of the proposed method was determined by deliberately changing some experimental conditions in a limited way. The flow rates of the mobile phase and mobile phase composition, column oven temperature, and detection wavelength were all adjusted individually and the HPLC chromatograms obtained compared to the chromatograms obtained under the standard conditions.

3.10.3.7.1 Effect on change in flow rate

A study was conducted to determine the effect of change in flow rate on the retention time (Rt) and area of the compounds. The flow rate was changed to 0.95, 1.0 and 1.1 ml/ min respectively. A standard solution of 20 ng/ ml of haloperidol or tramadol was prepared and analyzed using the variation in flow rates in comparison with the optimized method flow rate.

Acceptance criteria

The variation factor for haloperidol should not be more than 2.0 for variation in flow rate. The % RSD of peak asymmetry and retention time for haloperidol should not be more than 2.0% for variation in flow rate

(https://www.google.com/search?q=R.A.+van+Iterson+Drenthe+College+Emmen+Holland+for+www.standardbase.com&oq=R.A.+van+Iterson+Drenthe+College+Emmen+Holland+for+www.standardbase.com&aqs=chrome..69i57.2481j0j15&sourceid=chrome&ie=UTF-8).

3.10.3.7.2 Effect of change in mobile phase

A study was conducted to determine the effect of variation in mobile phase ratio by varying the ratio of the organic component in the mobile phase slightly. The organic phase of the mobile phase was varied by ±1 of the buffer: organic solvent mixture (v/v). A standard solution of 20 ng/ mL was

prepared and analyzed using the varied mobile phase composition in comparison with the optimized mobile phase in the method. A standard solution was prepared in triplicate and injected into the HPLC system. Where the acceptance criteria for the tailing factor of haloperidol was considered to not be more than 2.0 of variation in composition of mobile phase. The % RSD of the tailing factor and retention time of haloperidol or tramadol was not more than 2.0 for variation in composition of mobile phase (quansysbio.com/support/recovery-and-linearity/#).

3.10.3.7.3 System Stability

Sample solutions of haloperidol or tramadol were injected three times into the HPLC system as per test procedure at different temperatures of storage (4 and -20˚C) and time periods. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the % RSD of retention times, and peak areas from three replicate injections.

Acceptance criteria

The % RSD of the retention times of principal peak from three replicate injections for each standard solution should not be more than 2.0%

3.10.4 DETECTION OF HALOPERIDOL AND TRAMADOL IN PATIENT URINE SAMPLES

Haloperidol is highly plasma protein bound (up to 90%), extensively metabolized in the liver with approximately 1% of the original haloperidol dose expected to be eliminated via the kidneys in the urine (Mandal, 2019). Tramadol is not as highly bound to plasma proteins but is also metabolized in the liver and approximately 30% of the original dose expected in the urine (Vazzana *et al.*, 2015).

3.10.4.1 Collection of urine samples

Patient urine samples were collected from patients within a 24 hour period post-dose, in the morning and evening. The urine samples were centrifuged at 10 000 rpm for 15 min, the supernatant decanted and frozen at -70°C for storage until use. A volunteer urine sample (volunteer fasted overnight, on no medication) was also collected and treated in a similar manner to the patient urine samples.

3.10.4.2 Urine precipitation

Acetonitrile (6 ml) was added to each urine sample (2 ml) and vortexed for 10 min. Thereafter, the samples were centrifuged for 10 min at 5000 rpm. The supernatants were collected, filtered through

0.45 µm syringe filter and injected into the HPLC.

3.10.4.3 HPLC Method Establishment and Validation

The original validated RPC18-HPLC method used previously (Section 3.9.7) for the detection of and quantification of both haloperidol and tramadol in patient plasma samples was used for the detection and quantification of both compounds in patient urine samples (Table 3.2). Linearity of haloperidol and tramadol was established in the range of 0 to 50 ng/ ml. The quantification of haloperidol and tramadol in patient urine samples was performed by using the external standard method as used previously by Jain *et al*., 2011.

Table 3.2: RPC18-HPLC method for the detection and quantification of haloperidol and tramadol in patient urine samples

Parameter	Criteria
Column	Phenomenex Kinetex RPC18-HPLC Column with dimensions of 150 x 4.6 mm and particle size of 5 µm.
Mobile Phase	0.05M potassium phosphate buffer (pH 2.8): 0.05 M potassium phosphate buffer: acetonitrile (50:50 v/v)
Flow rate	1 ml/min
Detection wavelength	240 and 250 nm for haloperidol and tramadol, respectively
Injection Volume	20 µl
Run time	2 minutes
Column Temperature	20°C
Elution technique	Isocratic

3.10.4.3.1 Urine accuracy

For the accuracy of the established method in urine, the volunteer urine was spiked with a stock solution of 1 mg/ ml to make concentrations of 1 µg/ ml, 25 µg/ ml and 50 µg/ ml. These samples were tested for percentage recovery.

3.11 DATA ANALYSIS

Quantitative normally distributed variables were reported as means ± standard deviation (SD) and non-normally distributed variables as median and the interquartile range (IQR). Categorical

variables were expressed as proportions and percentages. Variables were assessed in the groups of the patients classified by the acute illness status. Parametric data will be assessed by t-test (two tailed) for paired and unpaired samples. Proportional odds logistic regression models were used to study the association between the delirium types and outcome (Gosho *et al*., 2023). Relationships between study variables (e.g. sleep quality and nurses' record of patient's sleep) were tested using Spearman's rank correlation coefficient for continuous data. The HPLC data were described in the form of mean, SD and %RSD using linear regression. STATA software version 13.0 (College Station, Texas 77845 USA) was used for the data analysis. $P < 0.05$ will be considered significant for all tests". *In vitro* data will be analyzed to determine the 50% inhibitory concentrations (IC_{50} values) using the "Mann- Whitney U-test with a p value of 0.05 considered significant" (Appendix N).

3.12 ETHICAL CONSIDERATIONS

The ethics application was submitted to the University of the Witwatersrand Human Research Ethics Committee (HREC) to get a waiver under the supervisor's name (Appendix H). Permission to carry out the research at the Charlotte Maxeke Johannesburg Academic Hospital has already been granted by the Chief Executive Officer of the Hospital (Appendix I). Patients' confidentiality was maintained at all times, anonymity upheld, patients were assigned research numbers and research codes, data collected and access were limited to the researcher and his supervisors only, and will be securely stored.

Information letters (patients) (Appendix J) and relatives (Appendix K) were written to explain the patient involvement in the study. Retrospective patient/relative consent form was also written in case the patient was too ill to consent (Appendix L, Appendix M). Consent letters were given to the prospective patients to read before agreeing to participate.

All participants were assured in writing of the confidential nature of the study by the researcher. No names or identifying characteristics were mentioned in the research, and care was taken to ensure participants were not identifiable. Six participants requested their data not to be included in the sleep study, which was done and removed from the main data. Participants were assured of their right to withdraw from the study at any time without prejudice. Data was kept securely. Data from the study was used for the purpose of this study, and as such, raw data was kept and placed in the custody of respective supervisors. Analyzed data was saved in computer files, password protected, and known only to the researcher and the supervisors.

3.13 SUMMARY

This chapter provided a detailed discussion of the research design and methods of the study, inclusive of the study population, sample and sampling methods, data collection and data analysis procedures that were followed to achieve the objectives of the study. Measures of validity, reliability are also highlighted, and ethical considerations and patients / relatives consent were presented.

CHAPTER FOUR

RESULTS AND DISCUSSION: DELIRIUM PATIENTS DESCRIPTION

4.1 INTRODUCTION

In the previous chapter, the methodology of the study was described. In this chapter, the statistical analysis employed to describe and synthesize the data, and interpretation of the results findings will be presented. The objective of the study was to evaluate the effect of haloperidol on stress hormones and oxidative stress on ICU patients with delirium and acute stress as well as perceived quality sleep of the patients and nurses. This was achieved using exploratory, descriptive and quantitative design. The study population included patients admitted to the General ICU at a University-affiliated, public sector and tertiary level hospital in the Gauteng Province in South Africa. A sample size of 94 (n = 94) patients was obtained. Data was collected by means of a data collection sheet to ensure standardize collection of information. Testing was done at the 0.05 ($p<0.05$) level of significance with a power of 95% confidence.

4.2 STATISTICAL ANALYSIS EMPLOYED IN THE STUDY

The results were described and analyzed using inferential statistics in accordance with study objectives. The results were summarized as frequency (f) mean (M), modes (Mo), median, standard deviation (SD), Standard error (SE) where applicable. Pearson's correlation coefficient (r), Chi-Square Test of Independence (X^2), paired t-test (t), Analysis of variance (ANOVA) and logistic regression, reported as odd ratio will also be used where applicable in this study. Descriptive statistics was employed to provide information on clinical data of the patients including age, gender, and length of hospital admission, disease severity and mortality. The cross tabulation and frequency distribution were used to summarize the clinical data of the patients. Bivariate analysis was employed to explain further the relevant data. To determine association between selected variables, contingency tables were used followed by inferential testing to achieve these. A one-way ANOVA (test of variance) was used to test the equal means within and across the group respectively. A two-way student t-test was used for mean score across categories of interest. From the patient outcome we created two groups of interest, being ICU Non-survival and ICU survivors. A two-sample t-test was then applied to determine statistical significance of the mean scores between these

groups. Fisher's exact test was used to determine relationships between the variables and to assign the test statistics. Testing was applied to various variables at different levels in order to determine the significant difference in mean scores.

4.3 DEMOGRAPHIC DATA

Demographic data was collected in order to describe the characteristics and relationships of the sample population and to enable comparisons between different categories with regards to the prevalence of the symptoms of delirium, anxiety, depression and post- traumatic stress disorder (PTSD).

4.3.1 Age, gender and sample size of the population

The total number of participants in this study was 94, with 46:48 ratios of females to males (Figure 4.1). The mean age was 45.49 years (SD = 17.45), the median was 44.5 years, and the mode was 28. The youngest participant was 19 years and the oldest was 85 years making the range 66 years (Figure 4.2). The majority of the participants were aged 18-35 years, where the black patients had the youngest mean age of 44 years, and the whites who had the oldest mean age of 62 years.

4.3.2 Race distribution of sample population

The majority of the sample participants were blacks n = 79 (84.0%), followed by n=14 Whites (14%) and Indian n=1 (1%) (Table 3.2). This is in line with the racial representation in the South African population, of which Blacks make up 77.7% of the total population (www.statssa.gov.za). However, a study by Wray, 2014 on racial integration in the Gauteng city-region reported that the black populations are the most predominant in the province. It has been reported that black patients who are discharged from ICU do not return for their follow up appointment compared to their White counterparts (personal discussion with ICU shift leader). Lack of transport fare, proxy collection of patient medications by the relatives were some of the reasons perceived to be responsible for their disengagement for follow up.

Characteristics	Overall age mean	Number of patients	Range	Percentage (%)
Age mean (range)	45±0.49	94	19-85	100
Female (n = 46)	46±0.76	46	47-66	48.94
Male (n = 48)	45±0.59	48	41-59	51.06

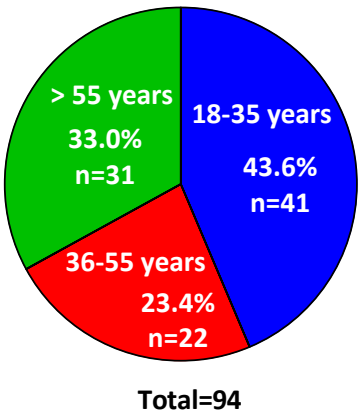


Figure 4.1: Demographic distribution of study population.

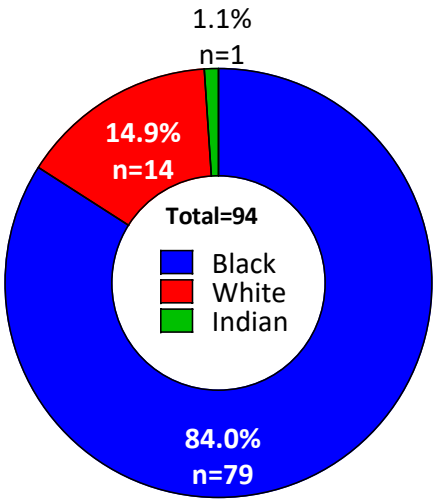


Figure 4.2 Race distribution of population of the patient population.

4.4 WORKING DIAGNOSIS OF ICU PATIENTS

All the patients in the study who were in the General ICU over the period of the study received advanced care and monitoring. The number of working diagnoses varies according to the patient's presentation. Patients with sepsis are the ones with highest presentations followed by suicidal attempt,' obstetric emergencies such as eclampsia were the least. For other working diagnosis refer to Figure 4.3.

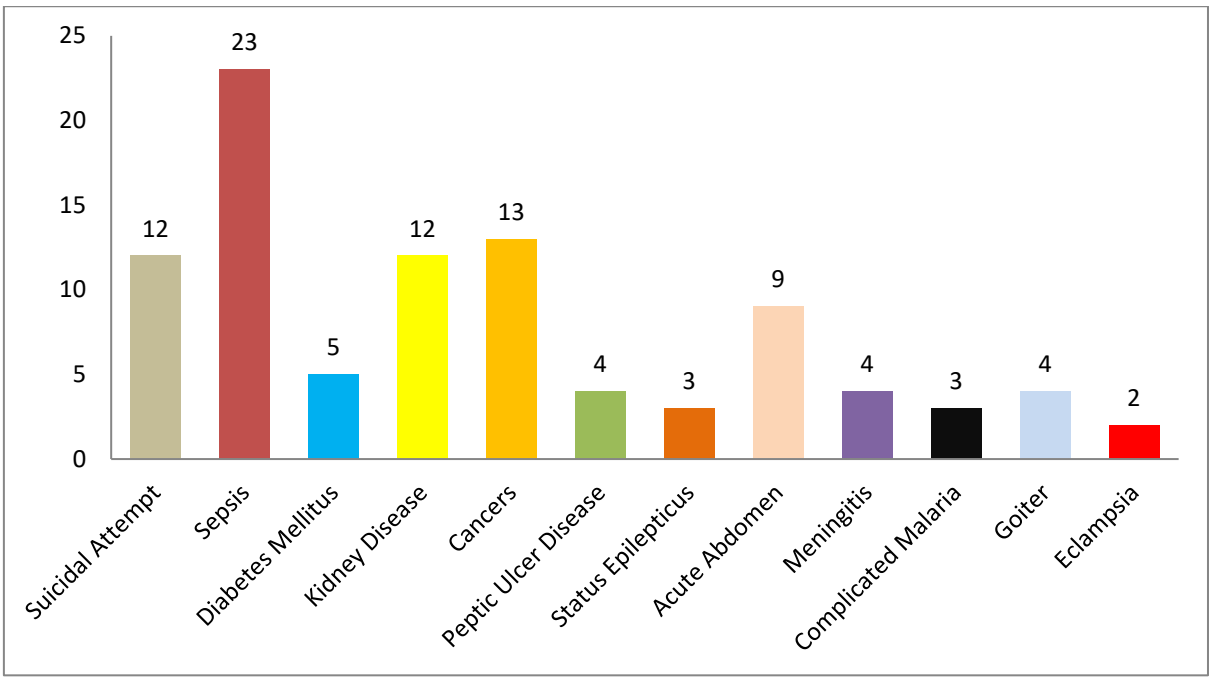


Figure 4.3: ICU patients working diagnosis.

4.6 SEVERITY OF ILLNESS

The level of severity of illness of critically ill ICU patients was determined using the SAPS II score (Table 4.4).

4.6.1 SAPS II score

Severity of critical illness as shown by the SAPS II score on ICU admission was 40.93 (SD 14.23) for the whole sample population (n=94), and the range was 70 on the scale. The minimum SAPS II score was 12 and the highest was 82 respectively. The average SAPS II score was higher among males than their female's counterpart, probably because males were the most reported to have comorbidities than females by many reviews (40.23 vs. 34.82). Details of comparison of SAPS II score with gender and reason for admission was shown on Table 4.1 below.

Table 4.1 Comparison of SAPS II score between ICUs by gender and reason for admission

Variables	Medical Emergencies (n=59)	Others (n=37)
Severity of Illness		
SAPS II Score	41.20 (12.11)	40.39 (11.50)
Gender		
Male	40.23 (12.13)	41.00 (12.40)
Female	34.80 (12.35)	39.50 (12.45)
Reason for Admission		
Medical emergencies	59.00	45.00
Others	39.43 (12.52)	33.60 (11.92)

4.6.2 SAPS II and Risk of Mortality

Calculation of the mortality risk for each patient was done on an online SAPS II score (New Simplified Acute Physiology Score) calculator was used to entered the variables obtained from patients and automatically obtained the results in percentages (http:www.sfar.org/scores2/saps2.html) Scores were divided into four groups according to their matched predicted risk of mortality scores. The grouping helps in monitoring varying predicted mortality risk when comparing with other ICU patients (Miranda *et al* 1996).

Of the total population (n=94), the SAPS II predicted mortality risk was 30.80% (Figure 4.4). Based on the categories, (the SAPS II score predicted mortality, it has total score of 0-163 each signifying predicted percentage mortality, for example 29 points on SAP II score predicted mortality is 10% and score of 77 indicated mortality of 90%) findings for predicted mortality risk showed that a higher number of patients was obtained in the SAPS II score groups from 0 to 19 (n=38), 20 to 39 (n=30) and 40 to 59 (n=11) points predicted mortality risk was respectively 38.0%, 30.0% and 11%, compared to lower number of patients in the SAPS II categories from greater than 80 (n=7) and 60 to 80 (n=8) points for predicted mortality risk was 7% and 8% respectively.

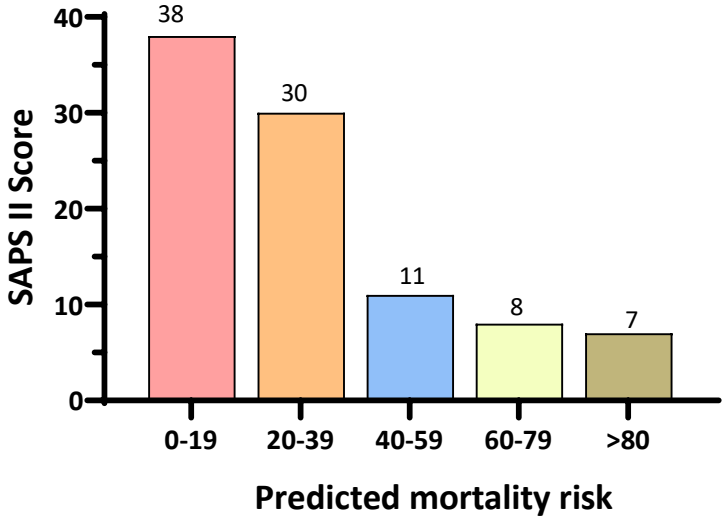


Figure 4.4 SAPS II score predicted mortality risk for the ICU population.

4.6.3 Precise ICU death rate

The actual observed ICU mortality rate for the whole population (n=94) was 14 (14.0%). Comparison of mortality among gender, we observed slightly higher 7 (14.0%) mortality among male patients as compared to female patients.

Note from severity of illness

- The total population for the study was 94. Severity of illness was determined by the total mean score, in this case it was 40.93 (SD 14.23) with minimum SAPS II score of 12 and highest score of 82.
- SAPS II score predicted mortality rate was 30.8% as against the precise ICU mortality rate of 14% (n=14)
- SAPS II score categories from 0 to 19 were higher in number compared to other categories (n=38) indicating that many patients survive ICU admission, while categories from 60 to 80 and greater than 80 have the least SAPS II score respectively.

4.7 REASON FOR ADMISSION

The reason for admission for the critically ill patients was categorized according to working diagnosis as presented (Table 4.8). Of the total sample population (n=94), the reason for admission of patients was higher (24.5%; n=23) for severe sepsis patients, and 13.8% (n=13) were cancer patients. Of all other patients, suicidal attempt patients were 12.7% (n=12), and with acute kidney

injury (AKI), which was also 12.7% (n=12) respectively. Other medical emergencies include acute abdomen 9.6% (n=9) and diabetes mellitus (Figure 4.1). Admission to the ICU was consistent in the order of severe sepsis and other medical emergencies (Figure 4.3). Of all the patients, the reason for admission was lower in the categories of complicated malaria (3.2%) and obstetric emergencies such as eclampsia (2.1%).

4.8 ICU PATIENT OUTCOME

For the purpose of analysis and explanation the outcome of critically ill patients were divided into two groups, i.e. ICU Non-survivors and ICU survivors Table 4.5.

There is no statistically significant difference in SAPS II score between ICU non-survivors and ICU survivors (p= 0.059; CI 0.34-4.33). In other words the SAPS II mean score for ICU non- survivors is not significantly higher than survivors mean, hence the two may have similar population groups (Table 4.2).

Table 4.2 SAPS II score by patient's outcome groups (ICU Non-survivors vs. ICU survivors)

Outcom2e variables	SAPS II score			
	Number	Mean	Standard Deviation	95% Confidence Interval
ICU Non-survival	14	**43.79**	16.20	36.93 - 55.64
ICU survival	80	39.17	13.79	33.10 - 43.01
Mean difference		**4.62**	**2.41**	**0.34 - 4.33**

Two way t-test; p=0.059

4.8.1 Relationships between variables for SAPS II score

In this section the relationships between variables and SAPS II scores, were demonstrated using Fisher's Exact Test to show the mean differences between the study groups (Table 4.10). For the fifteen SAPS II score items, only two variables were statistically significant ($p<0.05$). These variables include age and ventilation PaO_2/ FiO_2 ratio (item number Q1 and Q5) respectively. This means that the likelihood of these variables to have the same SAPS II score is relatively low. All the other variables showed no significance difference between their SAPS II score. In summary, the elderly and poor ventilation with oxygen indicated poor patient outcome Table 4.3.

Table 4.3 Comparison of SAPS II score items between ICU Non-survivors and ICU survivors

Variables	ICU Survivors (n = 80)	ICU Non Survivors (n = 14)	Mean difference scores	Fisher's exact test p- value
Q1. Age in years	5.710	7.000	1.290	0.001*
Q2. Systolic blood pressure	1.644	1.790	0.150	0.210
Q3. Heart rate (beat/ min)	1.400	0.547	0.853	0.305
Q4. Temperature (°C)	0.610	0.329	0.281	0.262
Q5. Ventilation PaO_2/ FiO_2	3.240	1.040	2.210	0.040*
Q6. Urine Output	0.403	0.093	0.310	0.110
Q7. Serum Urea	1.774	0.819	0.955	0.256
Q8. White Blood Cell Count	5.165	2.642	2.523	0.656
Q9. Serum Potassium	0.415	0.140	0.275	0.050
Q10. Serum Sodium	0.781	0.778	0.003	0.146
Q11. Serum Bicarbonate	1.608	0.385	1.223	0.123
Q12. Serum Bilirubin	4.484	1.260	3.224	0.067
Q13. Glasgow Coma Scale	2.291	0.779	1.512	0.305
Q14. Chronic Disease	2.937	0.698	2.239	0.235
Q15. Type of admission	1.249	0.594	0.655	0.095

Note = * statistically significant

4.8.2 SAPS II score and sub-items

Fisher Exact Test was also used in this section to see if there is significance difference between ICU Non-survivors and ICU survivors for total mean SAPS II score by sub-items. However, findings indicated age and ventilation to be statistically significantly different at sub-item level like ($p<0.05$) between ICU Non-survivors and ICU survivors (Table 4.4).

Table 4.4 Comparison of SAPS II score subitems score in ICU.

SAPS II	Outcome		Fisher's exact test p- value
	Non-survivors (n = 14)	Survivors (n = 80)	
Age in years	35.7%	43.0%	0.000 *
	28.6%	33.7%	
	7.1%	18.6%	
	0.0%	28.6%	
	0.0%	4.7%	
PaO_2/ FiO_2	0.0%	1.2%	0.040*
	14.3%	32.6%	
	42.9%	24.4%	
	42.9%	41.9%	

* = indicate statistical significance

Note from patient's outcome

- Among all the 15 variables of SAPS II items, only two were statistically significant ($p<0.005$). This being age (item Q1), and PaO_{2}/ FiO_{2} ratio (item Q5), respectively.
- The same sub-items were also statistically significant when compared among ICU Non- survivors and ICU survivors. This means that the older the patients the higher the mortality and also poor ventilation with oxygen may lead to poor outcome and subsequently death.

4.8.3 Length of stay in ICU

The average length of stay in ICU was 3.4 days, this result was skewed due to a small number of patients who had an extended (> 11 day) stay in ICU. The mode was two days; the median three days and the range 9 days. From the results it can be concluded that the majority of participants had a short stay in ICU. For easy categorisation, patients were divided into three groups: one to six days (1-6), seven to ten days (7-10), eleven days and above (> 11). It has been reported that patients that spend 11 days in the ICU are generally defined as a long-term ICU admission (Adamson, 2004). Eighty-nine participants (94.7%) stayed for one to six days; two (2.1%) stayed for seven to ten days, and three (3.2%) stayed for > 11 days (Figure 4.4).

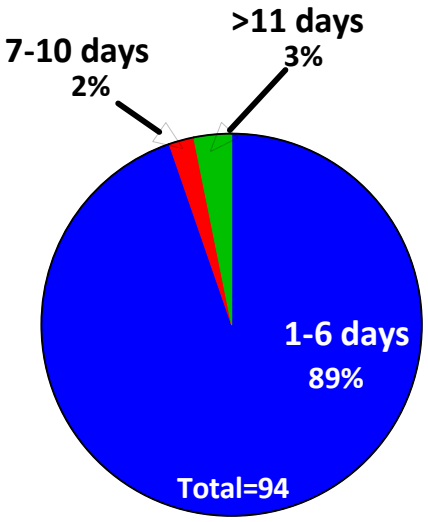


Figure 4.5 Length of stay by categories for the total ICU stay (n = 94).

Note for length of stay in the ICU

- The average length of stay was 3.4 days and range was 1 to 11 days for the study sample (n=94)
- Patients with medically complicated cases have slightly longer length of stay of 5.1 days, and by contrast, to the suicide attempted patients groups being 2.5 days.
- Majority (94.7%) of all ICU admissions length of stay was less than one week, which is considered as the short ICU stay category (Monsees *et al*., 2023). By comparison, only 3.2% of patients had a prolonged (> 11 days) length of stay, this showed a rapid turnover of patients in the general ICU of the hospital.

4.8.4 Mechanically ventilated patients

The number of patients who were mechanically ventilated was twenty-five (26.6%). The majority of the patients were not ventilated during their ICU stay n=69 (73.4%). This study included both ventilated and non-ventilated patients as the differences between the two groups have not been explored (Rotondi *et al.,* 2002). Most of the ICU studies described post-traumatic stress disorder among mechanically ventilated patients with the exception of some (Girard *et al*., 2007; Rotondi *et al*., 2002; Samuelson *et al*., 2007).

4.9 BIOCHEMICAL AND HAEMATOLOGICAL RESULTS OUTCOME

Patients were investigated at least twice in 24 hours while on admission. For easy analysis patients results were divided into two, namely, the day of admission (date 1) and any other day after the

first day of admission (date 2). This will give an insight into the patient's response to treatment. The median score of potassium and platelet count was noticed to be higher on the first day of admission (45.0 mmol/ l, date 1) and (200.5 x 10^9/ l, date 2) on the second day of admission respectively; although within the normal reference range values (3.3-5.3 mmol/ l vs. 137-373 x 10^9/ l). Other investigations showed relatively normal median values on date 1, except for creatinine level (137.5 µmol/ l, range: 47-90 µmol/ l); which shows a one third higher than normal results indicating that many patients suffered from severe renal impairment (Table 4.5).

Table 4.5 Haematological and biochemical results of ICU-patients (Date 1 vs. Date 2)

Test	Standard range	Date-1 median	Date-2 median	Range-1	Range-2	SD-1	SD-2
WCC	4.0-11.0 x10^9/L	65.5	30.0	38.0-93.0	19.0-94.0	38.89	40.51
Hb	♂:14.3-18.3 g/dL ♀:12.1-16.3 g/dL	10.0	10.0	10.0-10.0	10.0-10.0	1.00	1.00
PLT	137-373 x10^9/L	200.5	253.0	7.0-758.0	4.0-726.0	150.3	167.8
RBS	Up to 11.1 mmol/L	6.9	8.80	4.0-19.0	3.0-20.0	3.02	2.90
Urea	2.6-7.0 mmol/L	54.5	48.0	38.0-750.0	8.0-4057.0	282.7	64.72
Creatinine	47-90 µmol/L	137.5	108.0	8.0-4057.0	7.4-3394.0	488.8	414.2
Serum Na^+	135-147 mmol/L	138.0	142.0	120.0-156.0	129.0-157.0	6.26	5.59
Serum K^+	3.30-5.30 mmol/L	45.0	39.0	4.50-4.50	3.90-3.90	1.00	1.00
ALT	5-40 U/L	35.0	28.0	5.0-1097.0	10.0-1789.0	216.3	226.7
AST	5-40 U/L	55.0	35.0	10.0-1449.0	5.0-923.0	282.8	174.8
Albumin	35-52 g/L	28.0	23.0	5.0-60.0	3.0-47.0	9.86	6.96
Total bilirubin	0-21 µmol/L	20.0	18.0	3.0-342.0	3.0-300.0	54.55	52.33

4.9.1 Laboratory results and patient outcomes (ICU Non-survivors and survivors)

Hematological and biochemical results among ICU patients showed different outcomes. Four outcomes showed significant differences (albumin, platelet count, urea and creatinine) ($p<0.05$). Urea and creatinine results showed significantly higher counts indicating that many general ICU

patients suffered from severe renal impairment, systemic infection and relatively lower hepatic dysfunction cases. The remaining test results showed no statistically significant difference, indicating that the population may have similar laboratory results (Table 4.6).

Table 4.6 Comparison of laboratory outcomes (ICU Non-survivors and survivors).

[5]

Investigations	Reference Range	ICU Non-survivors (n=14)	ICU Survivors (n=80)	Mean difference value	Fisher's exact test p- value
Albumin	35-52 g/L	31.13	23.60	7.53	0.048*
Total Bilirubin	0-21 µmol/L	35.120	36.30	-1.18	0.377
WCC	4.0-10.0 x10^9/L	65.50	47.67	17.83	0.087
Platelet count	137-373 x10^9/L	275.90	230.46	45.44	0.000*
Haemoglobin	Male 14.3-18.3 g/dL, Female 12.1-16.3 g/dL	Equal value	Equal value	No difference	NA
RBS	Up to 11.1 mmol/L	7.68	8.78	-1.1	0.790
ALT	5-40 U/L	100..87	83.77	17.10	0.670
AST	5-40 U/L	140.27	90.15	50.12	0.774
Urea	2.6-7.0 mmol/L	175.50	76.0	99.50	0.000*
Creatinine	47-90 µmol/L	293.75	236.17	57.58	0.000*
Serum potassium	3.30-5.30 mmol/L	45.0	39.0	6.0	0.150
Serum sodium	135-147 mmol/L	138.29	142.68	-4.39	0.859

4.10 DRUG TREATMENT OPTIONS IN ICU

The ICU patients were administered various mediations to manage their pain, infections and related pathologies (Figure 4.6). ICU patients are known to be critically ill and prone to acquiring delirium (Andersen-Ranberg *et al*., 2022). Delirium patients are divided into two main groups based on the conventional classification into; agitated delirium, hypoactive and mixed type (rarely) (Figure 4.6). Over the five-month period of data collection (n=94) there were 36 agitated delirium patients. The majority of the cases were hypoactive delirium (60%), compared to 40% of the cases who had agitated delirium. All the agitated delirium patients were on antipsychotic, haloperidol, of which

only 20 patients received haloperidol in addition to other medications (risperidone, diazepam). The distribution of drugs used in the General ICU is presented in Table 4.7.

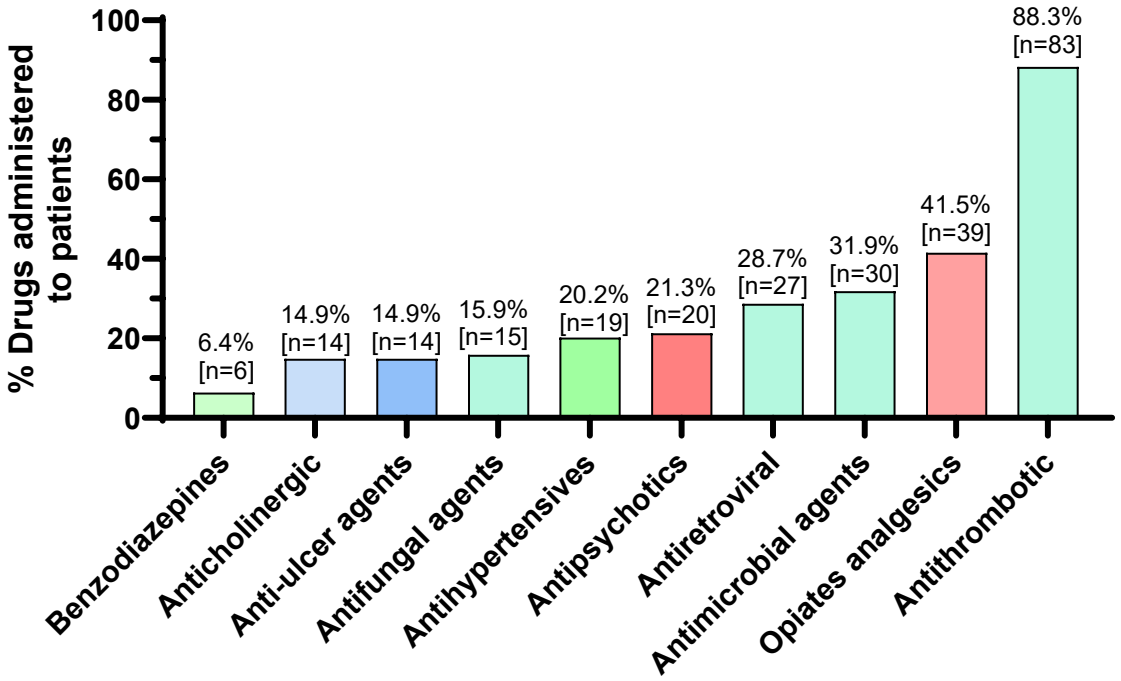


Figure 4.6 Drugs used and their distribution among General ICU patients.
Key = *indicated statistically significant

4.10.1 Antipsychotics and Benzodiazepines

These medications (antipsychotics, n=20, Benzodiazepines, n=6) were commonly used among ICU patients with delirium in order to relieve the symptoms of agitation and anxiety. Comparison among ICU categories showed that medically complicated cases had higher (20.0%) antipsychotic and benzodiazepines (6.0%) utilization compared to other emergencies (15.2% vs. 6.1%). The eclampsia, complicated malaria (22.2% vs. 11.1%) and status epilepticus (37.5%) have the significant utilization of these medications respectively, due to risk of recurrent seizures (Table 4.7).

Table 4.7 Pharmacokinetics of the antipsychotics, opiates and benzodiazepines drugs (Mauri *et al*., 2018, Siddiqui *et al*., 2023, Bauquier *et al*., 2024).

Agent	Haloperidol	Tramadol	Diazepam
Dose	Oral, IM, IV	Oral, IM, IV and rectal suppositories	Oral, IM, IV and rectal suppositories
Starting dose	0.5-1 mg twice a day	25-100 mg 4-6 times a day	2-2.5 mg
Half-life (hours)	12-38	Immediate release: 5-7 Delayed release: 12-48	30-56
Clearance	Hepatic	Hepatic	Hepatic
Common Side effects	Akathisia Dystonia Parkinsonism	Nausea Vomiting Serotonin syndrome	Anterograde amnesia Confusion Sedation
Effect on QT interval	Oral, IM: mild. IV: moderate	Nil	Nil
Orthostatic hypotension	Mild	Mild	Moderate
Anticholinergic	Mild	Mild	
Sedation	Mild	Mild	Moderate
Drug-drug interactions (CYP450)	CYP1A2 CYP2D6 CYP3D4	CYP2D6 CYP2B6 CY3A4	CYP 2C9 CYP 3A4 CYP 2B6
Special consideration	Mild effect on vital sign Worsen Parkinson symptoms	Some patient may have enhance or reduced opioid therapeutic or side effect	May lead to dependence if use improperly or excessively

Intravenous administration of haloperidol was the sole route of drug administration observed among the ICU patients at a starting dose of 0.5-1.0 mg (Table 4.7). However, haloperidol administration was primarily on the first day of admission except for those patients with persistent agitation at a much lower dose (1-3 mg). The drug was mainly administered by nursing staff on duty. Most patients had central venous lines in situ which are frequently used for drug administration. The details on pharmacokinetics of the antipsychotics, benzodiazepines and opiates analgesics is presented in Table 4.7

Diazepam together with other sedatives was predominantly used for sedation in ventilator and post-surgery patients. Diazepam administration requires close monitoring because of its unwanted side effects on hypoalbinaemic patients. This was observed in Table 4.6 where the results show

statistically significant differences among albumin count on date 1 of admission and date 2 respectively (p=0.048). This is consistent with the study by Buchanan *et al*., 1978 that found diazepam and morphine as the most dominant sedatives in their study (Buchanan *et al*., 1978). Other sedatives drugs used for critically ill patients in an ICU include propofol, inhalational anesthetics, alpha agonist and ketamine as reported by Wang *et al*., 2018. It is a well-known that inappropriate sedation may delay ventilation or winning, increase duration of admission, increase risk of pneumonia, bed sores and increase costs of treatment (Wang *et al*., 2018).

Table 4.8 Comparison between drugs used and ICU patients

Class of drugs	Number	Medical Emergencies (n=40)	Suicide Attempt (n =23)	Acute Abdomen (n=9)	Carcinomas (n=8)	Others (n=14)
Antipsychotics	20	10 (23.5%)	5 (5.8%)	2 (0.9%)	3 (1.2%)	1 (0.7%)
Opiates analgesics	39	21 (21.5%)	13 (7.7%)	7 (1.6%)	6 (1.2%)	7 (2.5%)
Benzodiazepines	6	3 (20.0%)	2 (7.7%)	1 (1.5%)	5 (6.7%)	5 (11.7%)
Antimicrobial agents	30	19 (25.3%)	8 (6.1%)	3 (0.9%)	5 (1.3%)	9 (4.2%)
Anticholinergics	14	2 (5.7%)	8 (13.1%)	4 (2.6%)	3 (1.7%)	1 (1.0%)
Antihypertensives	19	3 (6.3%)	12 (14.5%)	4 (1.9%)	1 (0.4%)	3 (2.2%)
Antithrombotics	83	39 (18.9%)	27 (7.5%)	9 (1.0%)	8 (0.8%)	14 (2.4%)
Anti-ulcer agents	14	6 (17.1%)	8 (13.1%)	-	7 (4.0)	12 (11.2%)
Antifungal agents	15	3 (8.0%)	10 (15.3%)	-	2 (1.1%)	1 (0.9%)
Antiretrovirals	27	7 (10.4%)	15 (12.7%)	3 (11.1%)	2 (0.6%)	1 (0.5%)

4.10.2 Opiates Analgesics

Opiate analgesics (n=39) are pain killers and used for moderate to severe pain among ICU patients (Mahmoud *et al*., 2022). Analysis among ICU patients showed that medically complicated patients utilize it more than any of the other groups 21 (21.5%) (Table 4.8). This is followed by suicide attempted patients 13 (7.5%). Similarly, acute abdomen patients and carcinoma related complications have the least utilization (1.6% vs. 1.2%) respectively. Treatment with single drug or

more than one drug (monotherapy versus combination therapy) with antipsychotic and opiates analgesic were presented (Figure 4.7).

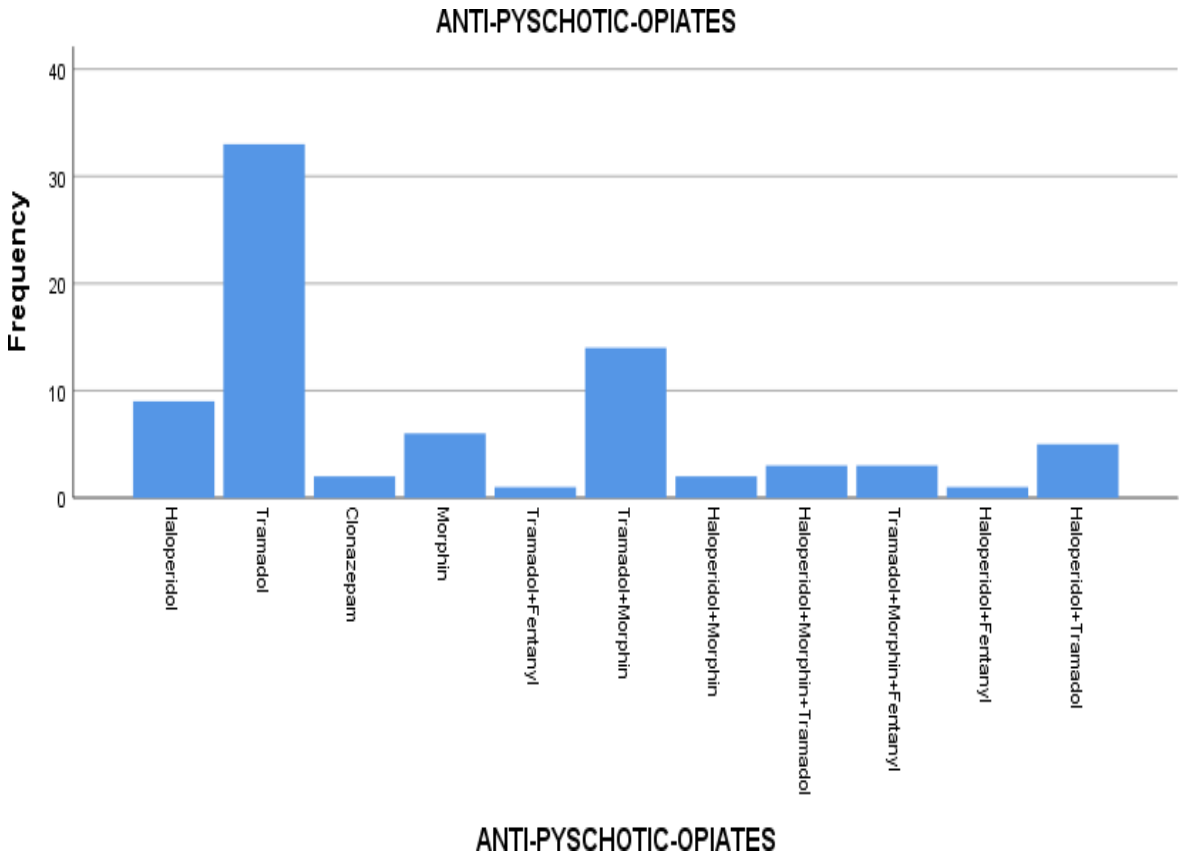


Figure 4.7 Distribution of opiate utilization among ICU patients.

Morphine was the predominantly used opiate for analgesia and sedation for patients on ventilator and post-surgery (Figure 4.6). However, continuous infusion of fentanyl was also administered to patients as an alternative opiates analgesic at a dose of 50-100 µg/h. Tramadol was often used enterally or intravenously for stable patients with mild to moderate pain.

4.10.3 Antimicrobial Agents

Of the fifty (n=40) medically complicated patients 19 (25.3%) were given antibiotics for prevention and treatment of infection compared to medical unit patients 8 (6.1%)(Table 4.8). Antibiotics administration is strictly regulated in the hospital through antibiotics stewardship policy and the reason for low utilization (personal conversation with unit manager). Only 3 (0.9%) patients with acute abdomen received antibiotics treatment and five others with carcinomas. Co-amoxiclav (Augmentin®) and ceftriaxone (Rocephin®) were the most common antibiotics employed. The high

incidence of Rocephin® usage reflects preference as post-operative antibiotics.

4.10.4 Other Drugs

Majority of the patients (n= 83) were exclusively on antithrombotic agents 83 (88.3%). This is due to ICU patients being prone to deep venous thrombosis (DVT). All the patients with carcinomas and other complications also received the antithrombotic medication, 0.8% (n= 8) and 14 (2.4%) respectively (Table 4.8).

Antifungal and anti-ulcer medications were more commonly used among medical patients 3 (8.0%) and 6 (17.1%) compared to surgery related emergencies respectively. Only 27 (28.7%) of the patients were on antiretroviral medications, which they received prior to admission to the unit. All the retroviral patients were on ABC+3TC+EFV (Abacavir, Lamivudine and Efavirenz) medication.

Antihypertensives (n= 19) were also prescribed more for suicide attempted patients 12 (14.5%) compared to medical and cancer patients 3 (6.3%) and 1 (0.4%) respectively. The higher incidence of Furosemide usage reflects a combination of the fact that many post- surgery and medical emergencies, especially AKI required diuretics. Digoxin was employed almost exclusively in post cardiac related complications as well as heart failure patients as presented in Table 4.8.

Pantoprazole was the predominant anti-ulcer medication used (14.4%) for prophylaxis and treatment of peptic ulcer disease and post-surgery bloating. Antiemetic; metoclopramide was also frequently used among cancer patients (n= 3) on cytotoxic drugs to reduce nausea and vomiting.

4.11 Sleep in the General ICU

4.11.1 Participants

During the study period 100 patients were eligible to partake in the study; six patients were ineligible, with 94 enrolled. The six participants were not included in the final analysis as they withdrew, unconscious or because of their inability to fluently communicate in English. Study participants were approximately 46 years old (mean, 45.49 years), remained on admission in the ICU for ≥24 hours and were able to provide a self-report of their sleep quality within the period of ICU stay (Table 4.9). All the 94 participants reported their sleep using RCSQ at least once. Participants provided data from one to 11 days, with a median of one day (IQR: 2-3) of sleep report per patient and more than 80% of participant were able to report on their sleep on second or third

day of the ICU for the whole participants (median, 85%, IQR: 40-66%).

Table 4.9 Other medications administered to critically ill ICU patients.

Class of drugs		Name of drugs used	
Antipsychotics	Haloperidol	Risperidone	
Opiates analgesics	Tramadol	Morphine	Fentanyl
Benzodiazepines	Diazepam	Clonazepam	
Antimicrobial agents	Amoxicillin-clavulanic acid	Ceftriaxone	Azithromycin
	Metronidazole		
Other drugs	Ketamine	Propofol	
	Pantoprazole		
	Fluconazole		
	Clexane and Vitamin K		
	ARVs (ABC+3TC+EFV)		
	Atropine		
	Dexamethasone		

4.11.2 Patient's perception about their own sleep

The patient's report on the rating of how they had slept the previous night varied widely (Table 4.10). Some reported good night sleep while others stated that they had not slept throughout the night (Table 4.10). The average sleep quality during ICU stay was described as poor by the patients with median scores for each of the variables of sleep quality below 50 mm (Figure 4.8). Table 4.10 shows variation in patients according to how they responded to RCSQ questionnaire statements. The total sleep score ranged between 7-86 mm (mean, 45.79). Twelve patients (12.8%) scored a total sleep score of 25 and below (i.e. very poor sleep) and 35 patients (37.2%) scored a total score of over 75 (i.e. very good sleep) (Figure 4.8).

Table 4.10 Findings of Richard Campbell Sleep evaluations from questionnaire.

Question		Range	Mean	Std. Deviation
1.	Sleep depth	5-100	45.54	17.99
2.	Sleep latency	5-80	43.83	17.74
3.	Awakenings	5-100	45.38	19.32
4.	Returning to sleep	5-100	46.54	18.83
5.	Sleep quality	5-100	47.66	19.67
Total sleep quality		7-86	45.79	17.08
6.	Noise level	10-90	50.51	17.56

Scoring of patients (*n* = 94). Ranges and means for the individual questions for the total sleep score are indicated.

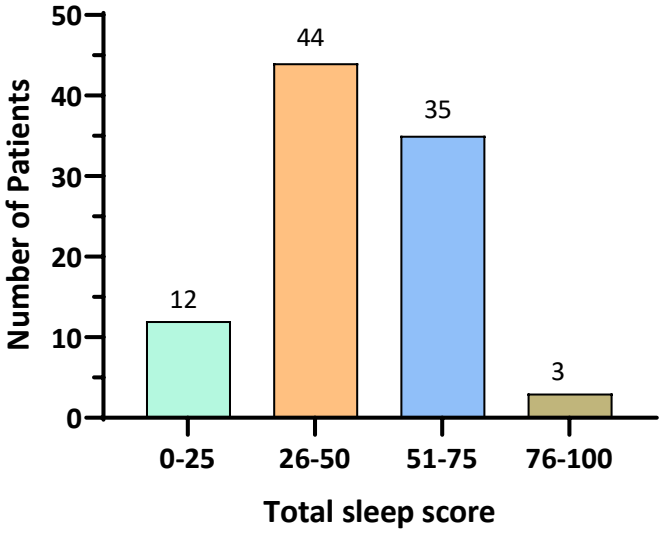


Figure 4.8: Patients divided into four groups based on the total sleep score (*n* = 94).

4.11.3 Duration of treatment in the ICU versus total sleep score

A comparison between the patients who were treated for five days and above in the ICU (n = 11) with those treated for a short period i.e. four days and below (n=83). After comparison, a statistically significant difference was found in the total sleep score [treated for five days and above (mean, 32.73) versus treated for four days and below (mean, 47.48) respectively] (*P* = 0.007).

4.12 Antipsychotics and sedatives administration

Seventy nine of the 94 patients (84.0%) were administered some form of antipsychotics or sedatives during their ICU stay. On comparing the group receiving antipsychotics or sedatives (n=73) and the

group not receiving antipsychotic or sedative (n=21), it was noted that the one not receiving antipsychotics or sedative had slept better and had a higher total sleep score (mean, 45.64) than the one receiving antipsychotics ($p< 0.05$). Other medications administered to the patients and their effects on sleep, is shown on Table 4.11.

Table 4.11 Common drugs administered in the ICU and their effects on sleep architecture (Bourne *et al., year*).

Drugs used in the ICU	Effect on sleep	Mechanism of action
Benzodiazepines	Reduce REM & SWS	Act by stimulating γ-Aminobutyric acid type A receptor stimulator
Phenytoin	Increase sleep fragmentation	Block influx of neuronal calcium
SSRI	Reduce REM, TST & SE	Increased serotonergic action
β-Blockers	Reduce REM, Insomnia & nightmares	CVS β-blockade by lipophilic agents
Amiodarone	Nightmares	Not known
Corticosteroids	Insomnia, reduce REM & SWS	Low melatonin secretion
Opioids	Reduce REM & SWS	μ-Opioid receptor stimulation
NSAIDS	Reduce TST & SE	Inhibition of prostaglandins production
Dopamine	Insomnia, reduce REM & SWS	D_2- & α_1-adrenoreceptor stimulation
Quinolones	Insomnia	*γ*-Aminobutyric acid type A receptor inhibition

Abbreviations: SWS = slow-wave sleep, REM = rapid eye movement, NSAIDS = Non-Steroidal Anti- Inflammatory Drugs, SSRI = Selective-Serotonin Reuptake Inhibitors, TST = Total Sleep Time, SE = Sleep efficiency.

4.13 Noise level in the ICU

The patients rated their own experience about the noise level in the ICU during the course of the study. The noise level rating ranged from 10-90 (mean, 53.39), indicating a rather low noise level in the ICU. A statistically significant correlation was noted between the total sleep score and the noise level (r= 0.568; p= 0.000).

4.14 What factors disturbed or promote patients' sleep during the night?

All 94 patients in the study were asked; if there was anything in particular that disturbed their sleep during the night. One patient (1.1%) stated that there was nothing in particular that disturbed their sleep. Thirty-five of the patients (37.2%) stated that the cause of their disturbed sleep was discomfort experienced in the form of pain. Twenty patients (21.3%) mentioned procedures while four patients (4.3%) indicated 24-hour light exposure. The remaining thirty-four patients (36.2%) point out noise in the form of beep noise, outside cubicle noise, mainly from the staff conversation as well as noise arising from their own cubicle, mainly from the fellow-patients who had for instance moaned or snored. On the other hand, the majority of the patients 58.5% (55) said they tend to sleep well when the environment is quiet while 28 patients (29.8%) only sleep well, whenever the light is off. Other patients mentioned medications (analgesic, sedatives and antacid) as well as comfortable positions as their main sleep promoters respectively, 13.8% (n =13) and 4.3% (n =4). Other factors were also mentioned by the patients (Table 4.12).

Table 4.12 Patients reported factors that deter or promotes sleep.

Promoters of sleep	
Pharmacological	Medications such as: sedatives, pain killers, antacids.
ICU environment	Quietness, deem light, beeps.
Nursing care	Comfortable position, wash/bath.
Psychological	Prayers, family visit.
Deterrents to sleep	
Clinical condition and care	Physical and emotional pain, discomfort, inability to communicate, dry mouth, clinical symptoms.
ICU environment	Light on, beep noise, outside cubicle noise (from staff), inside cubicle noise (from other patients), during ward rounds (doctors).
Psychological	Unfamiliar environment, fear, overthinking.
Procedures and instruments	Intubation, nasogastric tubes, urethral catheters, tracheostomy suctioning.

4.15 Nurses' perceptions of the patients' sleep

The nurses' bedside record was reviewed on how they report the patient's sleep. The report was summarized as: "good sleep" "moderate sleep" and "poor sleep" (Figure 4.9). A comparison was made between the patients' perception of their own sleep and the nurses' perception of the patients sleep. In comparison the patients' perception (mean, 45.76) and the nurses' (mean, 45.76). No statistically significant difference was found. A correlation analysis shows some degree of correlation (r= 0.532; p= 0.000).

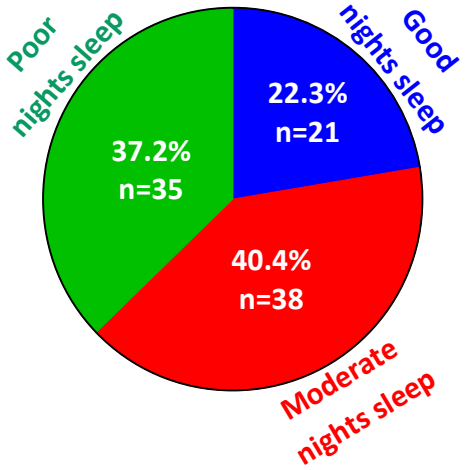


Figure 4.9: Nurse's sleep record of Genera ICU patients based on their perception.

4.16 Prior stressors

About twelve patients (12.8%) were admitted in the ICU, with a history of suicide attempt (organophosphate poisoning). This indicated that they had prior stressful event in their lives prior to admission. These stressors were varied among the patients and ranged from relationship issues, unemployment and family pressure. No patients personally revealed previous history of psychiatric illness or receiving medications from any institution because of psychological stress problems. We assumed this may probably be due to stigma associated with mental illness.

A Tables 4.12, 4.13, 4.14 provides information on the symptoms of anxiety, depression and PTSD among the General ICU patients, and the effect of the variables gender, race and length of ICU stay. Mean value, Standard Deviations, Range and Median values are provided.

4.17 Anxiety symptoms among General ICU patients

A total of 45% of the patients have the symptoms of anxiety (SE: 0.05; CI: 0.95-1.65) using a cut-off

mark of eight on Hospital Anxiety Depression Scale (HADS) which have a mean score of 8.1 (SD, 4.5) and a range of 21 (Table 4.12). Among the patients with symptoms of anxiety 15% had a score of 12 and above, which indicated that patients had a case of anxiety according to Scragg *et al*, 2001. White patients had the highest number of anxiety symptoms (56.4%) compared to black patients (43.6%), with no significant difference between the two races (p= 0.65). Patients between the ages of 18-35 years, namely, younger patients showed more signs of anxiety than older ones (44.8%). Middle aged patients (36-55 years) had a prevalence of 42.6% while the older patients (>55 years) showed the lowest prevalence of anxiety symptoms (10.6%), there is significant difference between the groups (p< 0.02). The trend in Females were more likely to have anxiety based on the study compared to their male counterparts (54.3% vs. 45.7%), with no significant difference between the groups (p= 0.43)(Table 4.13).

Table 4.12: Prevalence of anxiety symptoms in patients

Variable		% Patients with anxiety symptoms	Standard Error	95% Confidence interval
Gender	Male	42.7	0.075	1.40-1.71
	Female	51.3	0.068	1.34-1.61
Age	18-35 years	42.8	0.126	0.88-1.39
	36-55 years	40.6	0.115	0.61-1.07
	>55 years	10.6	0.070	0.30-0.60
Race	Black	40.6	0.067	1.13-1.40
	White	53.4	0.044	0.98-1.16
	Indian	No observation		

Table 4.13: Anxiety symptoms (Mean, SD, Range, Median).

Variables		Number	Mean	SD	Range	Median
Gender	Male	48	6.9	3.5	0-20	7
	Female	46	7.1	4.7	1-21	9
Race	Black	79	9.7	4.6	0-19	8
	White	14	7.8	4.4	0-21	8
	Indian	1	3.2	2.2	2-7	5
Length of stay	1-6 days	89	9	5.3	0-21	9
	7-10 days	2	7	4.2	2-10	8
	>11 days	3	4	2.3	1-8	5

4.18 Depressive symptoms among General ICU patients

A total of 25% (SE0.03; CI 0.86-1.77) of the patients showed symptoms of depression with 8 as the cut-off point on HADS depression scale (Wu *et al*., 2021). The mean score of 6.5 (SD 3.73) and range of 16 was the acceptable cut-off mark on this scale (Table 4.14; 4.15). Only 9% of the depressive patients score the cut-off point of 11 and above which denote depression according to Scragg *et al.,* 2001. White people showed the highest prevalence of depression (73.8%) compared to blacks (20.2%) with a significant difference (p< 0.04). On the contrary, other previous studies found males to have the highest prevalence of depression than women (53.6% vs. 40.4%) respectively (p< 0.05). Patients older than 55 years (42.5%) had the highest prevalence of depressive symptoms compared to younger patients (26.6% vs. 30.9%) (p= 0.42).

Table 4.14 Demographic profile of patients diagnosed with depressive symptoms

Variables		% patients with depressive symptoms	Standard Error	95% Confidence interval
Gender	Male	53.6	0.118	1.28-1.77
	Female	40.4	0.56	1.39-1.62
Age	18-35 years	23.6	0.020	0.57-1.43
	36-55 years	29.9	0.95	0.77-1.15
	>55 years	40.5	0.98	1.70-1.90
Race	Black	20.2	0.096	1.01-1.41
	White	73.8	0.043	1.06-1.23
	Indian	No observation		

Table 4.15: Demographic profile of patients diagnosed with depressive symptoms (Mean, SD, Range, and Median)

Variables		Number	Mean	SD	Range	Median
Gender	Male	48	6.5	3.2	0-16	6
	Female	46	6.1	3.6	1-16	7
Race	Black	79	8.2	3.8	0-16	7
	White	14	5.3	3.4	1-14	5
	Indian	1	4.1	2.1	1-16	4
Length of stay	1-6 days	89	7.6	4.7	0-17	8
	7-10 days	2	6.4	3.8	0-11	6
	>11 days	3	6.2	3.0	1-14	7

4.19 PTSD symptoms among General ICU patients

A total of 38% (SE 0.049; CI 0.49-1.46) showed symptoms of PTSD (Table 4.16). According to Scragg *et al* 2001 a total score of eight and above on ETIC-7 scale is the cut-off marks for diagnosing PTSD (mean 5.9; SD 5.3; range 21) as presented in Table 4.5. PTSD was more prevalent among blacks. compared to the whites in this study (79.8%). Females had the higher rate of PTSD than males, with females having prevalence of 48.9% (SE 0.056) compared to males (51.1%) (SE, 0.068). The older patients showed the highest prevalence of PTSD symptoms (28.7%) compared to the younger population (8.5% vs. 20.2%) respectively (p= 0.23) (Table 4.16).

Table 4.16 PTSD symptoms (Mean, SD, Range, and Median)

Variables		Number	Mean	SD	Range	Median
Gender	Male	48	6.5	3.2	0-16	6
	Female	46	6.1	3.6	1-16	7
Race	Black	79	8.2	3.8	0-16	7
	White	14	5.3	3.4	1-14	5
	Indian	1	4.1	2.1	1-16	4
Length of stay	1-6 days	89	7.6	4.7	0-17	8
	7-10 days	2	6.4	3.8	0-11	6
	>11 days	3	6.2	3.0	1-14	7

4.20 Anxiety, Depressive and PTSD symptoms among General ICU patients

In this study, the anxiety symptoms were most prevalent among the patients (45%); followed by 38% of the patients with PTSD symptoms and 25% with symptoms of depression. Depressive symptoms are least compared to anxiety and PTSD symptoms respectively (45% vs. 38%) (Figure 4.10). Some ICU studies reported factors like prolonged use of sleep-inducing medications, prolonged admission and longer duration of mechanical ventilation as the main risk factors of ICU depression (Du *et al*., 2023). In this study the majority of the patients (97.9%) stayed in the ICU for a short period of time of 1-6 days (Table 4.17), and only 26.6% of these patients received mechanical ventilation. This may explain why we have lower depressive symptoms compared to anxiety and PTSD respectively.

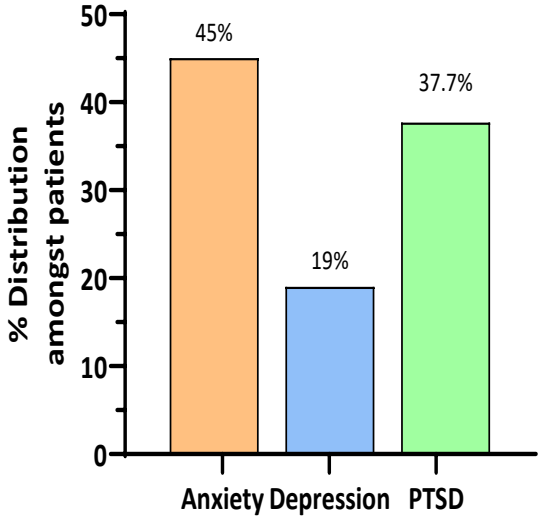


Figure 4.10 Distributions of anxiety, depression and PTSD symptoms among patients.

Table 4.17 Distribution of Anxiety, Depression and PTSD symptoms among ICU patients

Variables		% of patients with anxiety symptoms	% of patients with depression symptoms	% of patients with PTSD symptoms
Gender	Male	25.0	9.0	19.0
	Female	20.0	10.0	18.7
Race	Black	33.0	15.0	32.0
	White	12.0	4.0	5.0
	Indian	0.0	0.0	1.0
Age	18-35 years	13.0	5.0	8.0
	36-55 years	14.0	22.0	19.0
	>55 years	27.0	27.0	27.0

4.21 Items on PTSD checklist

The checklist has seven direct questions, the questions focus mainly on intrusive behaviour of the patients and avoidance of activities, places or persons that will remind them of their previous experience of post-traumatic disorder. We asked 94 patients on their previous experience in the ICU based on these seven questions and audio recorded their respective responses (Table 4.18).

Table 4.18 shows that 48% of the patients are having intrusive experience (item-1), 70% had withdrawal symptoms (sum of item-6 and item-7). Seventy-seven percent have negative feelings or anxiety (sum of Item-3 and Item-5) when reminded of their time in ICU. Thirty-four percent were having nightmares associated with ICU admission (item-4).

Table 4.18 Items on PTSD checklist/ scale

Item on PTSD scale	Number of patients with PTSD symptoms	Total % of patients with PTSD symptoms
1. Had upsetting thoughts or images about your time in ICU?	45.12	48%
2. Experienced “flashbacks” when you are reminded of your time in ICU?	34.78	37%
3. Felt upset when you are reminded of your time in ICU?	28.20	30%
4. Had bad dreams or nightmares about your time in ICU?	31.96	34%
5. Felt anxious or unwell when reminded of your stay in the ICU?	44.18	47%
6. Tried not to think about, talk about, or have feelings about your time in ICU?	41.36	44%
7. Avoid activities, people or places that remind you of the ICU	24.44	26%

CHAPTER FIVE

RESULTS AND DISCUSSION: HPLC ANALYSIS

5.1 METHOD DEVELOPMENT FOR HALOPERIDOL, TRAMADOL AND SOTALOL

Introduction

After the two compounds (Haloperidol and tramadol) were accurately weighed and separated they were then stirred to dissolve completely. The solutions were then filtered through 0.45 µm syringe filters (section 3.9.8.1). These stock solutions were used for further dilutions (stock 1 solution). From the original stocks, a further 1000 ng/mL of both compounds were made to at least 5 ml and these stocks (stocks 2 solutions) were used for further dilutions for the respective standard curves. Stocks were also prepared using volunteer (blank) plasma and compounds extracted as described in section 3.9.6.

The method was developed according to section 3.9.8 in the method section. According to section 3.9.8 the standard (100 and 150 ng/ ml) was tested with mobile phase (Methanol: Acetonitrile; 50:50 v/v) and no peak was obtained for haloperidol and tramadol respectively (Figures 5.1 and 5.3). The optimized method for haloperidol and tramadol according to section 3.9.8 was achieved at a flow rate of 1 ml/ min, wavelengths of 240 and 250 nm, injection volume of 20 µl and run time over 2-3 minutes for haloperidol and tramadol respectively. A retention time of 1.8 and 1.4 minutes for haloperidol and tramadol was obtained respectively for the optimized method (Figures 5. 2 and 5.4).

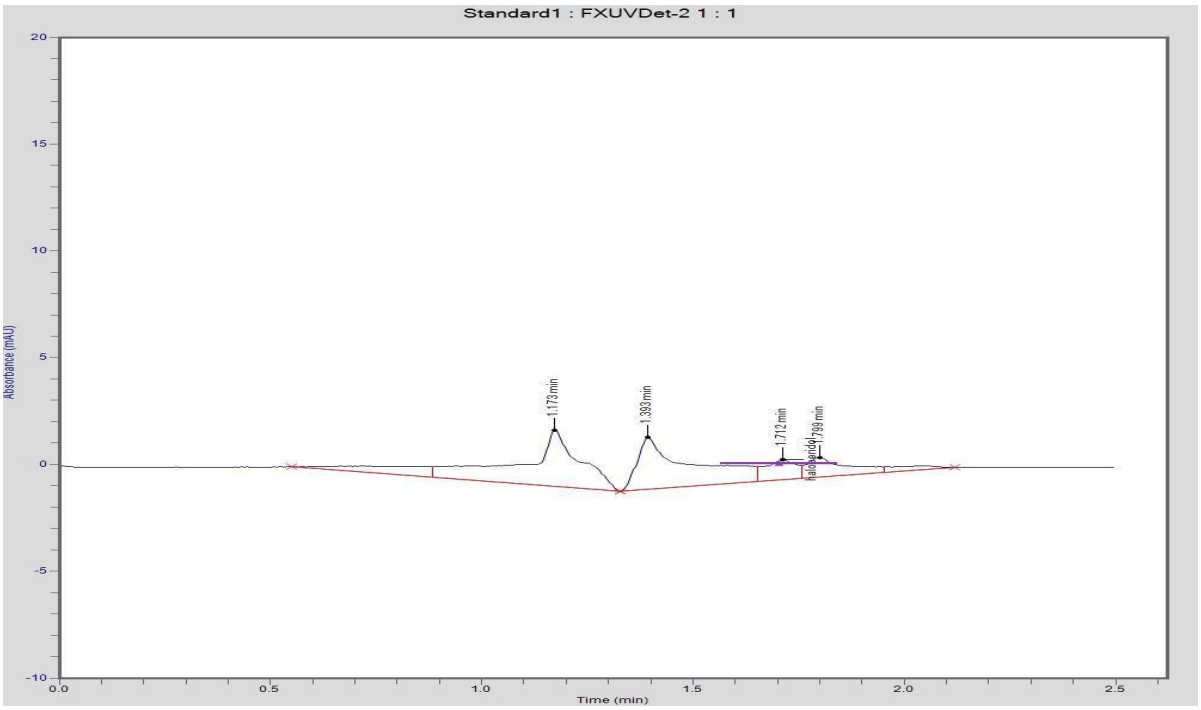


Figure 5.1: Chromatogram for Haloperidol (1 ng/ml) in mobile phase (Methanol: Acetonitrile; 50:50 v/v) (Rt = 1.8 min).

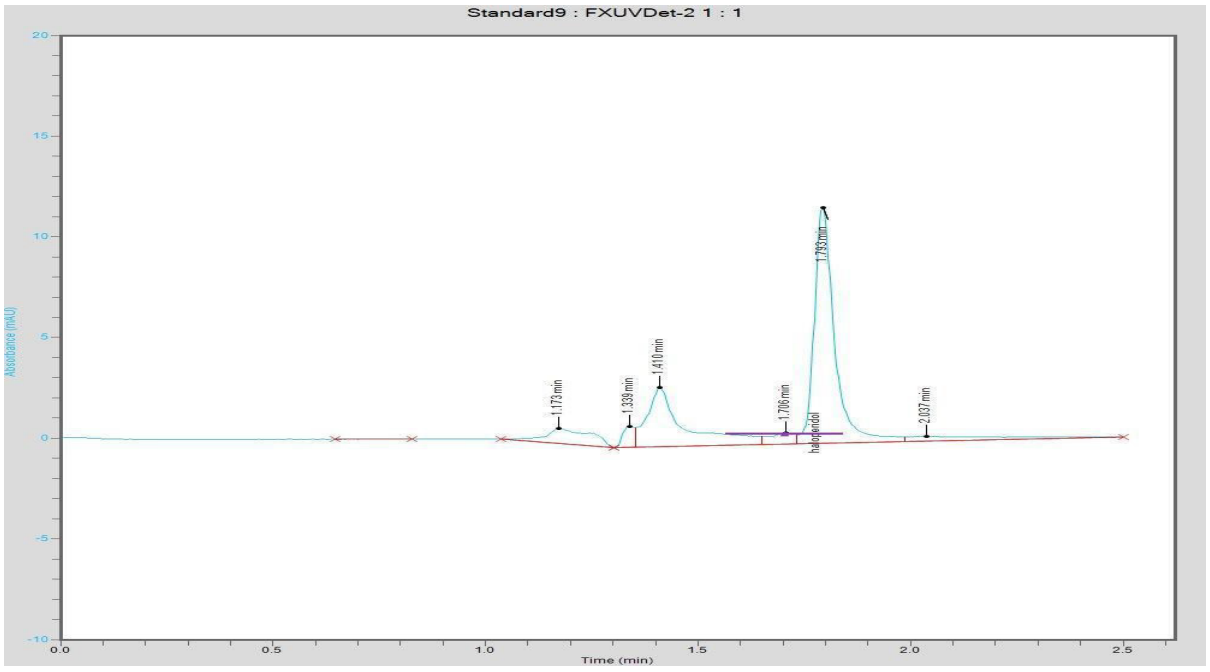


Figure 5.2: Optimized HPLC chromatograms of haloperidol (150 ng/ml) in mobile phase (Methanol: Acetonitrile; 50:50, v/v), (Rt = 1.80 min).

5.1.1 Method Development For Tramadol

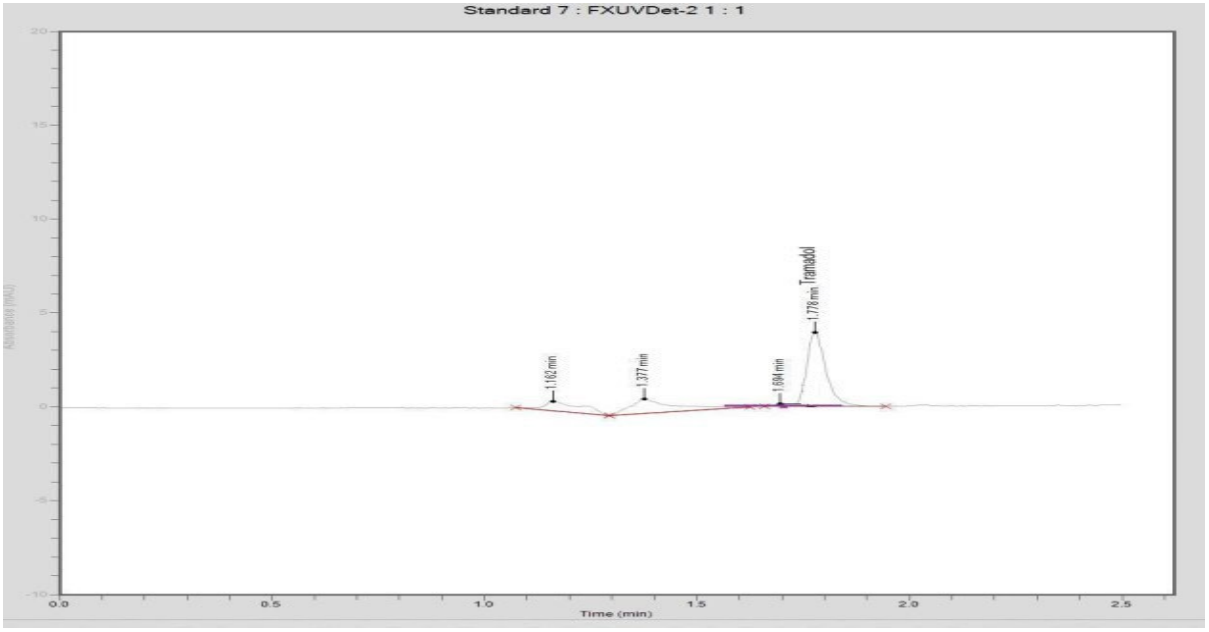


Figure 5.3: Chromatogram for tramadol (50 ng/ml) in mobile phase.

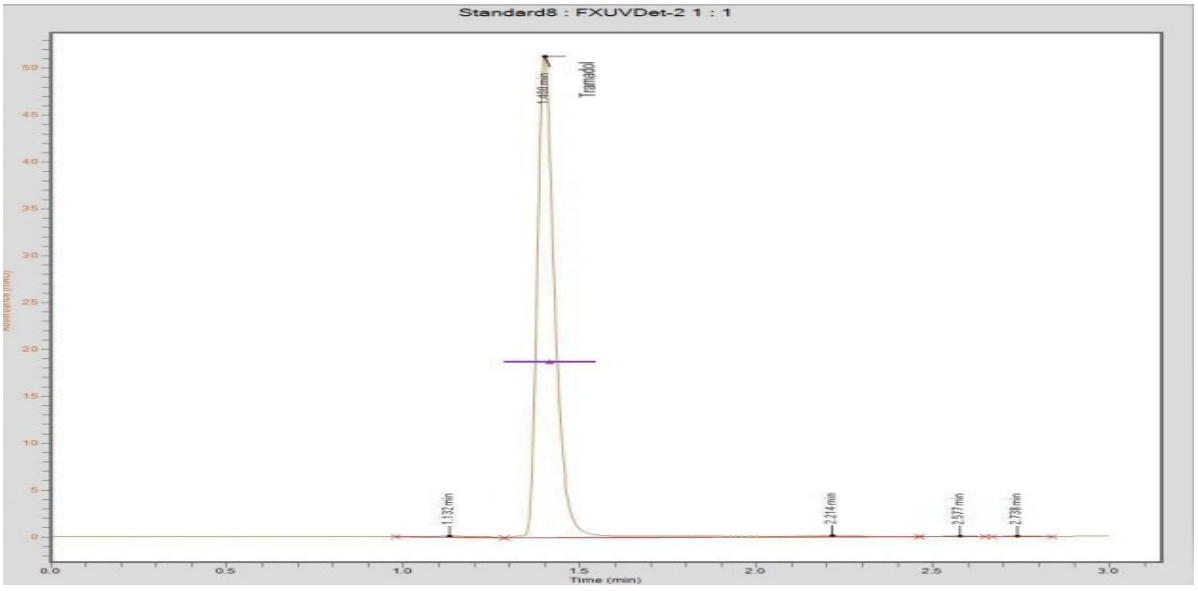


Figure 5.4: Optimized HPLC chromatograms of tramadol (100 ng/ml) in mobile phase (Rt = 1.4 min).

5.2 METHOD VALIDATION FOR VOLUNTEER AND BLANK PLASMA IN MOBILE PHASE

Method validation process provides evidence that the method is consistent and reliable and can be used for routine analysis. Using the optimized chromatographic conditions (Figure 5.1, 5.2, 5.3 and 5.4) for samples as prepared in mobile phase, the developed HPLC method was validated with

respect to linearity, precision, accuracy, reproducibility, LOD and LOQ as per the ICH guidelines in both mobile phase, volunteer and blank plasma (Kazusaki *et al*., 2012).

5.3 Haloperidol in mobile phase

5.3.1 Linearity

The linear regression model encompasses the ability to predict values for a dependent variable whilst the independent value is known. The model determines the relationship between the dependent and independent variable. The linearity is usually reported by means of the coefficient of correlation (R) or coefficient of determination (R^2), respectively. A desired value of 0.999 for the R^2 value indicates zero error and is evident of a linear relationship between the dependent and independent variables (https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf.)

In this method validation, the calibration curve was constructed after running freshly prepared haloperidol standards in mobile phase that consisted of acetonitrile and 6.8 g of potassium monophosphate, 1 ml of acetic acid and pH was adjusted with orthophosphoric acid to pH of 2.8 (50:50 v/v). A linear relationship was observed between peak area and concentration (Figure 5.5). The linearity of haloperidol solution was obtained over the range of 1-200 ng/mL. The linear regression equation was found to be Y = 285.11X + 3364 with a coefficient of determination (R^2) of 0.9869 and retention time (R_t) of 1.8 min (Figure 5.2). The limit of detection (LOD) was found to be 8.70 ng/ mL while the limit of quantification (LOQ) was 26.40 ng/ mL respectively (Table 5.1)

Table 5.1: Linearity and regression parameters with precision values

Parameters	Values
Linear range, ng/ ml	1-200
Regression equation	Y = 285.11X + *3364*
Limit of detection (LOD), ng/ ml	8.70
Limit of quantification (LOQ), ng/ ml	26.40
Coefficient of variation (R^2)	0.9869

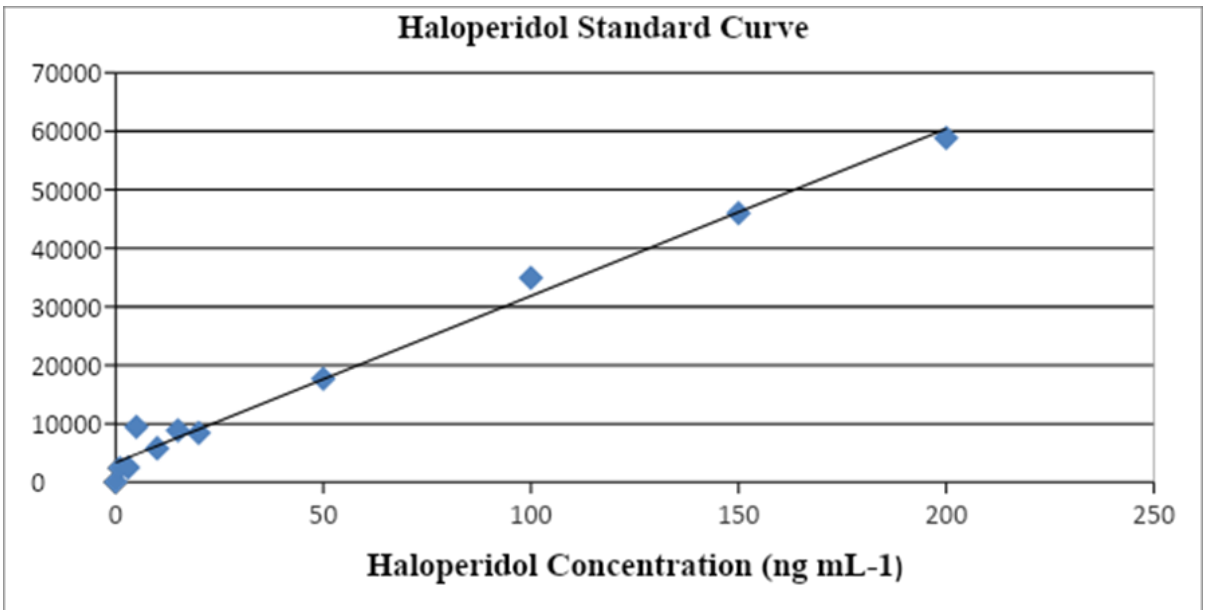


Figure 5.5: Calibration curve of haloperidol in mobile phase (Acetonitrile: Phosphate buffer (pH 2.8); 50:50 v/v). Each analysis was repeated 3 times (n=3).

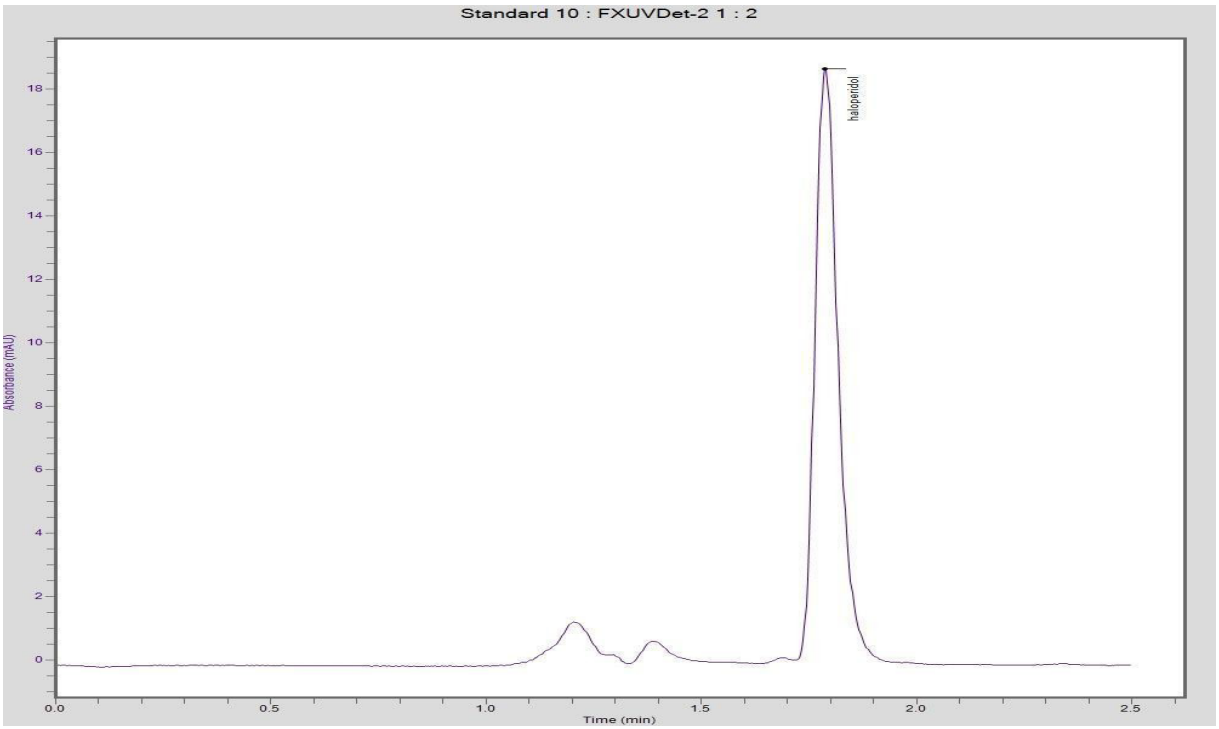


Figure 5.6: HPLC chromatogram of haloperidol in mobile phase (10 ng/ mL). Rt = 1.79 min.

The drug is well detected and separated even at lower concentration, as shown in Figure 5.6 above

5.3.2 Precision

Precision refers to the closeness between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed condition. Precision may be expressed as: repeatability, intermediate precision and reproducibility.

The precision was assessed by analyzing haloperidol in mobile phase in three different concentration levels in triplicate as 20, 50 and 200 ng/ mL. The results of repeatability (intraday precision) and intermediate (interday) precision were expressed as % Relative Standard Deviation (%RSD). The intraday and interday precision study of the developed method confirmed the adequate sample stability and method reliability where most %RSDs are within acceptable values of ≤ 2%; however, %RSD at the lower concentration is slightly more as expected, because at lower concentration the variability in data is usually greater (Ngwenya *et al*., 2023). It is noted that areas under curve (AUC) obtained from chromatograms at the same concentrations are lower on the next day, which might indicate slight instability as shown in Table 5.2. The stability of the drug spiked at three QC levels evaluated for: short term (6 h), freeze thaw (3 cycles), and long term (3 days). The results (Table 5.2) showed that the HPL was stable in human plasma for about one year when stored in the frozen state (-80°C).

Table 5.2: Interday and intraday precision of haloperidol in mobile phase (n=3).

Concentration (ng/mL)	Intraday precision		Interday precision 3 days	
	Mean Area ± SD	%RSD	Mean Area ± SD	%RSD
20	10820.10 ± 283.50	2.62	8241.90 ± 54.50	0.66
50	42211.60 ± 77.50	0.18	38342.70 ± 115.90	0.30
200	88575.40 ± 1042.30	1.18	81040.90 ± 474.90	0.59

Summary note:

- The precision results showed adequate sample stability and method reliability.
- Values are slightly lower on the next day, i.e. 23.8, 9.2 and 8.5% loss for the 3 concentrations tested, respectively on the next day.
- %RSDs were overall less than 2% which is in line with acceptable criteria of ≤ 2% (https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf)

5.4 Tramadol in mobile phase

5.4.1 Linearity of Tramadol

The linearity of tramadol is illustrated in Figure 5.7 below. The linear regression curve showed that the compound was linear between the following values; 1-200 µg/mL with a regression equation of Y = 0.0028X – 2.3769 and R^2 value of 0.999. Details of other variables related to the linearity of haloperidol in mobile phase (Table 5.3).

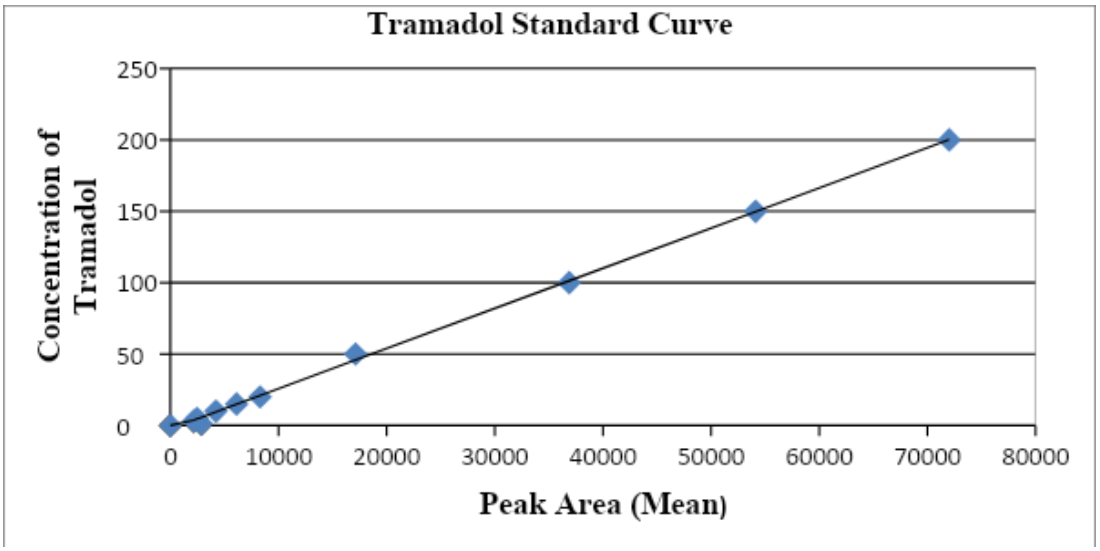


Figure 5.7: Calibration curve of tramadol in mobile phase (Acetonitrile: Phosphate buffer (pH 2.8); 50:50 v/v). Three runs were repeated for each sample to form a standard curve. This was done for the 10 concentrations.

Summary note:

- The compound is well separated in the mobile phase as shown in the chromatogram Figure 5.8.

Table 5.3: Linearity and regression parameters with precision values for Tramadol

Parameters	Values
Linear range, ng mL^{-1}	1-200
Regression equation	Y = mX + c
Limit of detection (LOD), ng/mL	8.69
Limit of quantification (LOQ), ng/mL	26.36
Coefficient of determination (R^2)	0.999

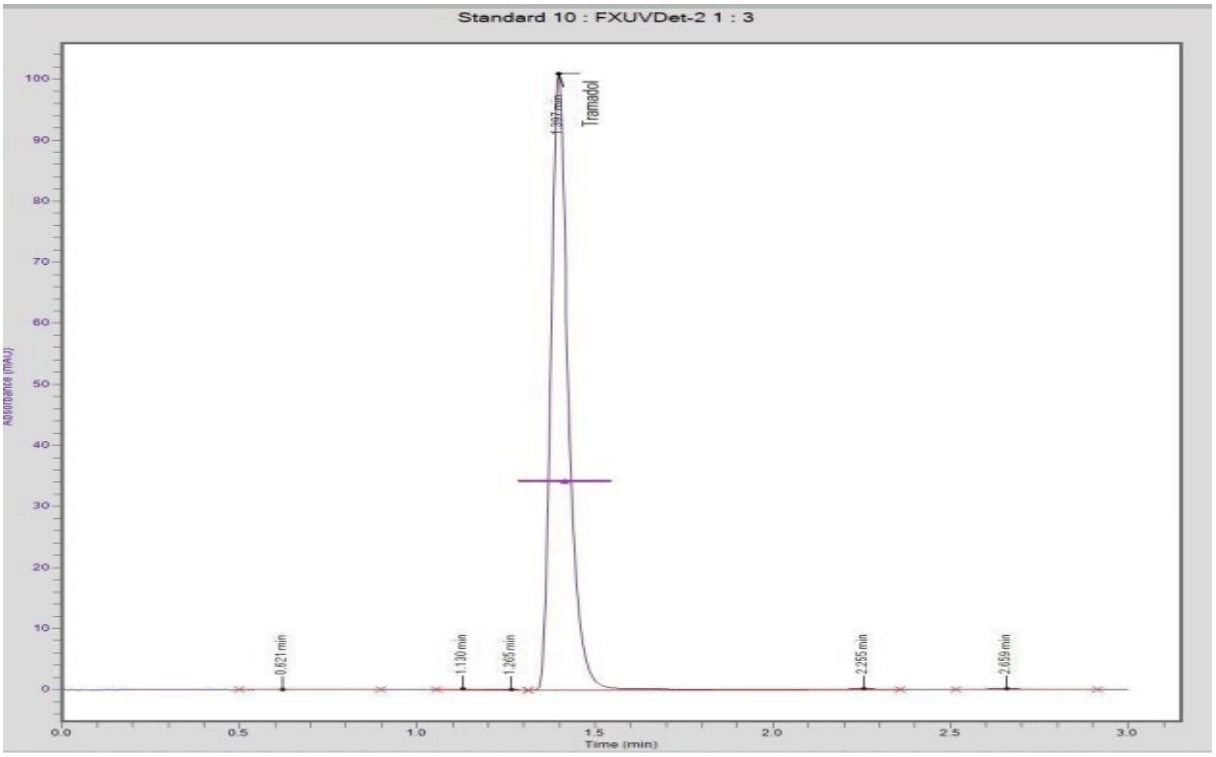


Figure 5.8: HPLC chromatogram of Tramadol in mobile phase (Methanol: Acetonitrile; 50:50 v/v)

5.4.2 Precision

Table 5.4 reports on the mean and the percentage relative standard deviation data of Tramadol. The acceptance criteria for precision of data are expressed in %RSD with a value of ≤ 2% indicating good precision of the method.

Table 5.4: Precision of proposed method of Tramadol in mobile phase

Concentration (ng/mL)	Intraday precision		Interday precision 3 days	
	Mean Area ± SD	%RSD	Mean Area ± SD	%RSD
20	41541.75±117.35	0.28	37788.46±30.75	0.08
50	204488.60±282.46	0.23	115378.60±357.63	0.31
200	87699.10±3590.05	0.93	354208.50±2680.77	0.80

Summary note:

- The %RSD of the three concentrations did not significantly change despite variation in concentration. The slight variation in AUC is attributed to the ongoing loss of the samples probably due to a long period of storage
- Intraday precision %RSD for lower and higher concentration of 20 and 200 ng/ ml were slightly higher than the intermediate concentration of 50 ng/ ml

5.5 Internal standard

Instrumental signals or experimental procedures may produce changes in the results; an internal standard may be used to compensate for such challenges. The internal standard must be able to be analyzed with the same mobile phase but be separated from the analyte tested, so there must be no interference between the two (Marson *et al*., 2020). This method is based on the fact that the ratio of concentration between the analyte and internal standard remains constant, even when instrument drift or sample is lost during treatment or not completely introduced in the instrument. For example, there is a need to use the internal standard method when determining the concentration of compounds in plasma samples. This is due to the extraction procedure and loss in the compound that might occur (Yasir *et al.,* 2017). Under those circumstances, both the sample and the internal standard are lost, but the ratio of concentrations (analyte concentration/ internal standard concentration) remains constant, and so the ratio of signals remains constant (analyte signal/ internal standard signal) (Yasir *et al*., 2017).

5.5.1 Linearity of the internal standard (Sotalol)

The linearity of sotalol is illustrated in Figure 5.9. Other details with respect to the regression equation, LOD and LOQ parameters are shown in the Table 5.5.

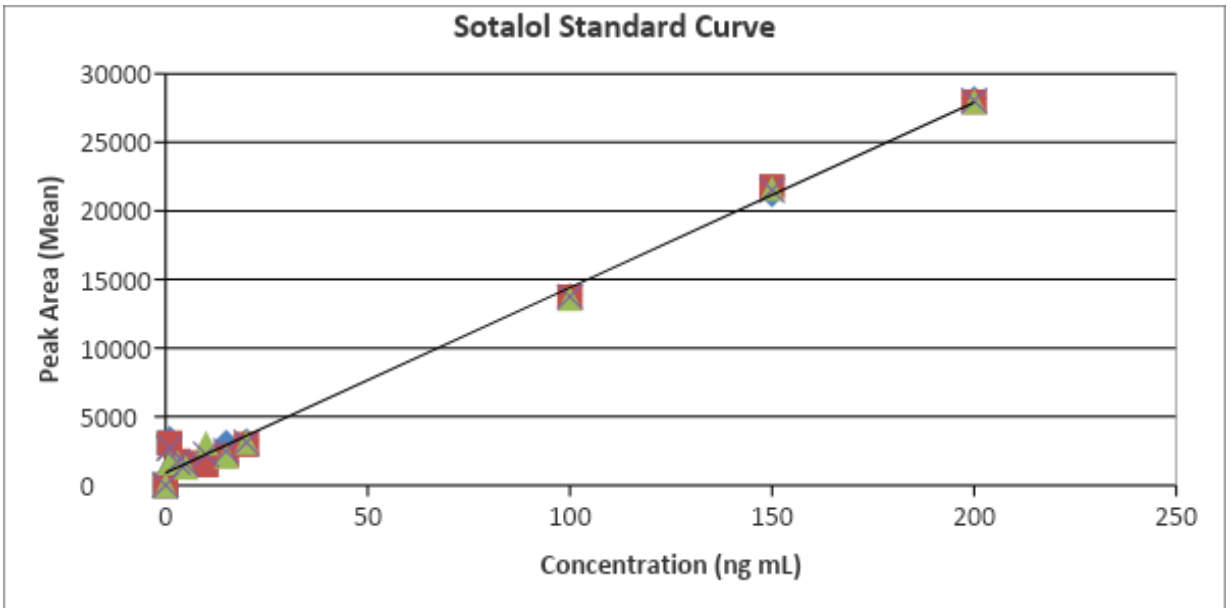


Figure 5.9: Calibration curve of sotalol in MP (Methanol: Acetonitrile; 50:50 v/v). This experiment was done in order to determine the concentration of sotalol to add to plasma samples so as to detect the two compounds easily, and to test whether sotalol does not mask the peaks for haloperidol or tramadol.

Table 5.5: The linearity and regression parameters of sotalol with precision values

Parameters	Values
Linear range, ng mL^{-1}	1-200
Regression equation	Y = mX + c
Limit of detection (LOD), ng/mL	0.02
Limit of quantification (LOQ), ng/mL	0.06
Coefficient of determination(R^2)	0.9945

Summary note:

- Sotalol was linear over the specified concentrations. A concentration of 87.5 ng/ mL was used further for addition to all plasma samples as it was easily detected and did not interfere with the other 2 compounds (Figure 5.10-5.12).
- The internal standard was well separated in mobile phase and volunteer/blank plasma.

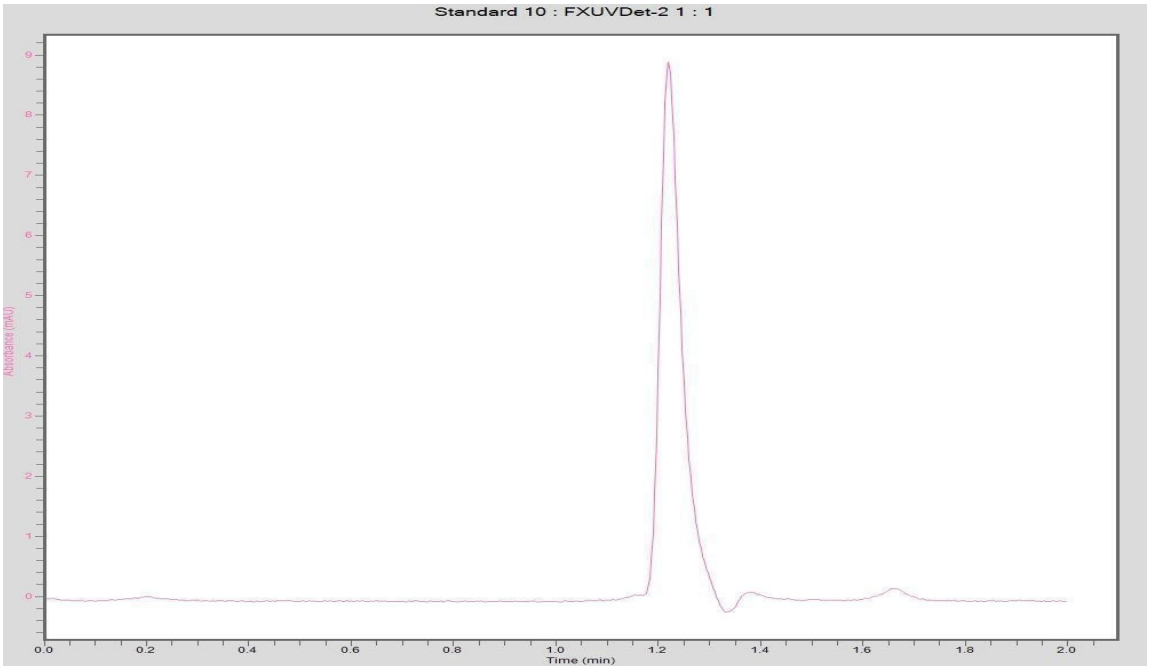


Figure 5.10: HPLC chromatogram of sotalol (100 ng/mL), Rt = 1.2 min in MP (Methanol: Acetonitrile; 50:50 v/v)

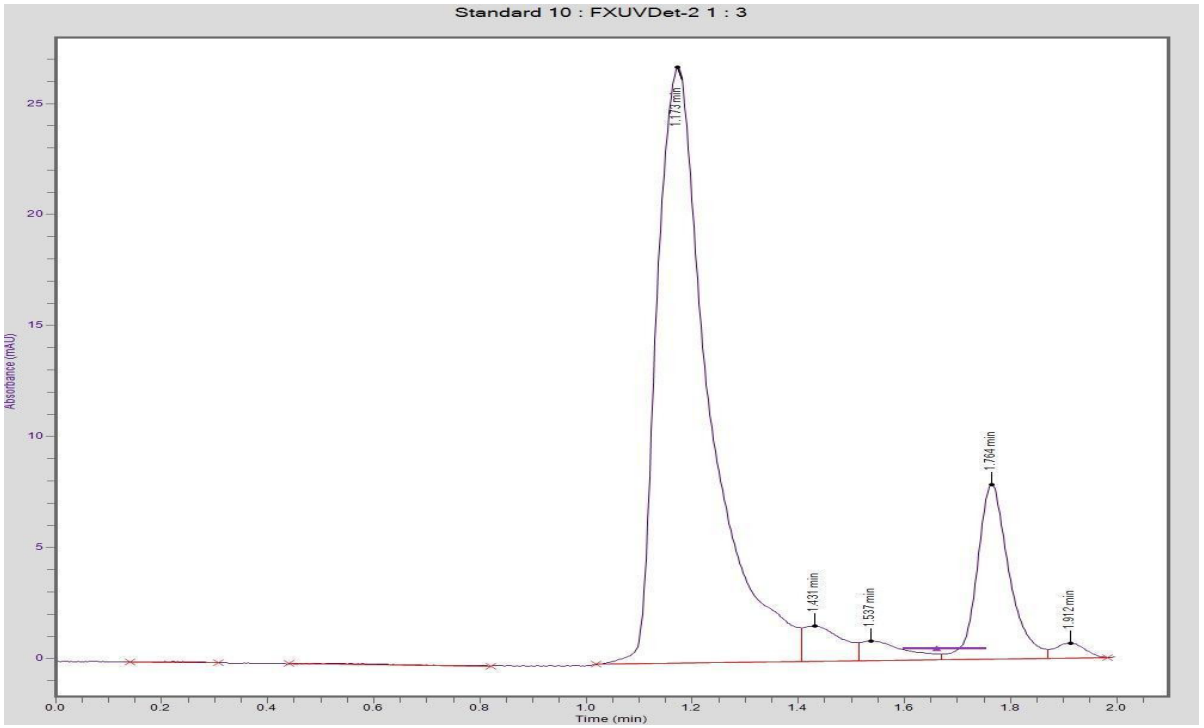


Figure 5.11: HPLC chromatogram of sotalol (100 ng/mL) (Rt = 1.2 min) and haloperidol (Rt = 1.8 min) in MP (Methanol: Acetonitrile; 50:50 v/v)

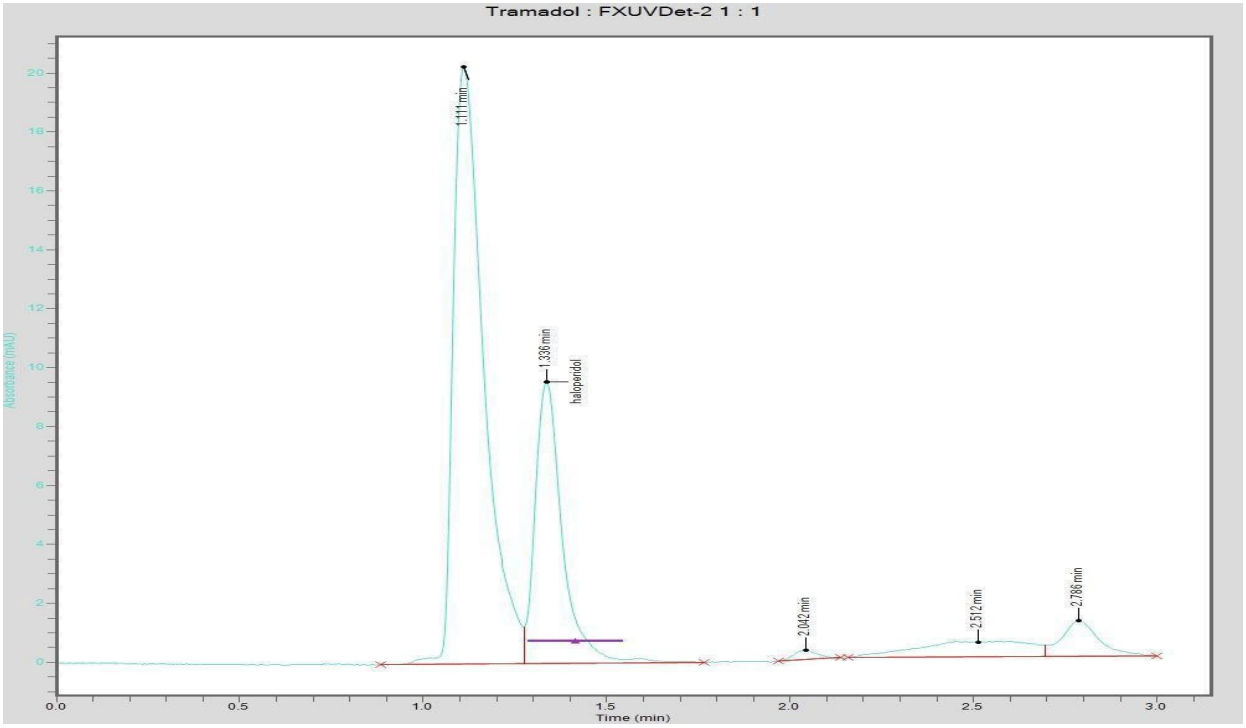


Figure 5.12: HPLC chromatogram of sotalol (Rt = 1.2 min) (3 ng/mL) and tramadol (Rt = 1.4 min)

5.6 Haloperidol in volunteer plasma

5.6.1 Linearity of Haloperidol

Like in the mobile phase, fresh standards were prepared, spiked in volunteer/blank plasma and extracted from the plasma. The results obtained were used to construct a standard curve (with a resultant linear equation) between peak area ratio of haloperidol to sotalol and concentration ratio of haloperidol to sotalol. The retention time for haloperidol was 1.79±0.9 minutes and 1.2±0.4 minutes for sotalol respectively. Information on the linearity of haloperidol in volunteer plasma, standard curve and haloperidol chromatogram in plasma is presented in Table 5.6 and Figure 5.12.

Table 5.6: Linearity and regression parameters with precision values in volunteer/ blank plasma of haloperidol

Parameters	Values
Linear range, ng mL^{-1}	1-200
Regression equation	Y = mX + c
Limit of detection (LOD), ng/mL	0.005
Limit of quantification (LOQ), ng/mL	0.14
Correlation coefficient (R^2)	0.986

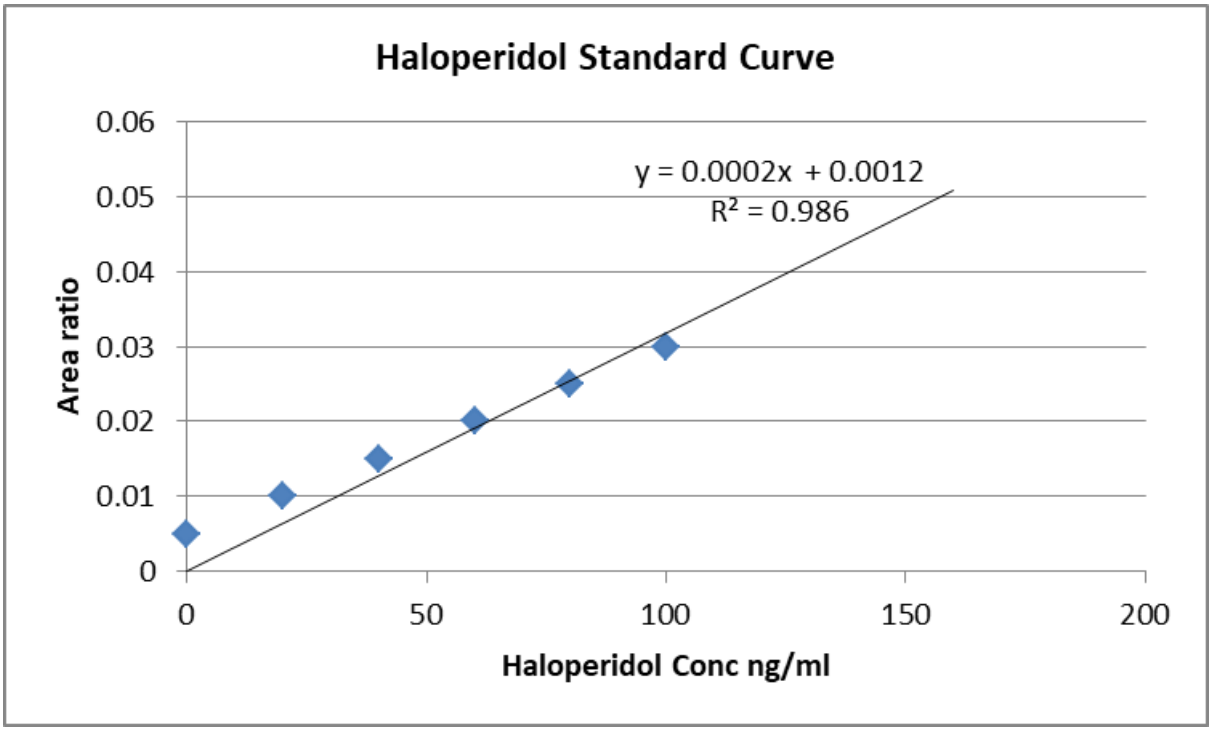


Figure 5.13: Calibration curve of haloperidol in plasma.

Summary note:

- The sotalol peak showed slight tailing while the haloperidol peak was symmetrical, but much lower in height. The two peaks were adequately separated (Figure 5.14).
- The internal standard sotalol (87.5 ng/ mL) was added to compensate for losses during extraction procedures.
- The ratio of concentration between the internal standard and the analyte added was kept constant i.e. 87.5 ng/mL sotalol was added to all plasma samples but the concentration of haloperidol was varied.

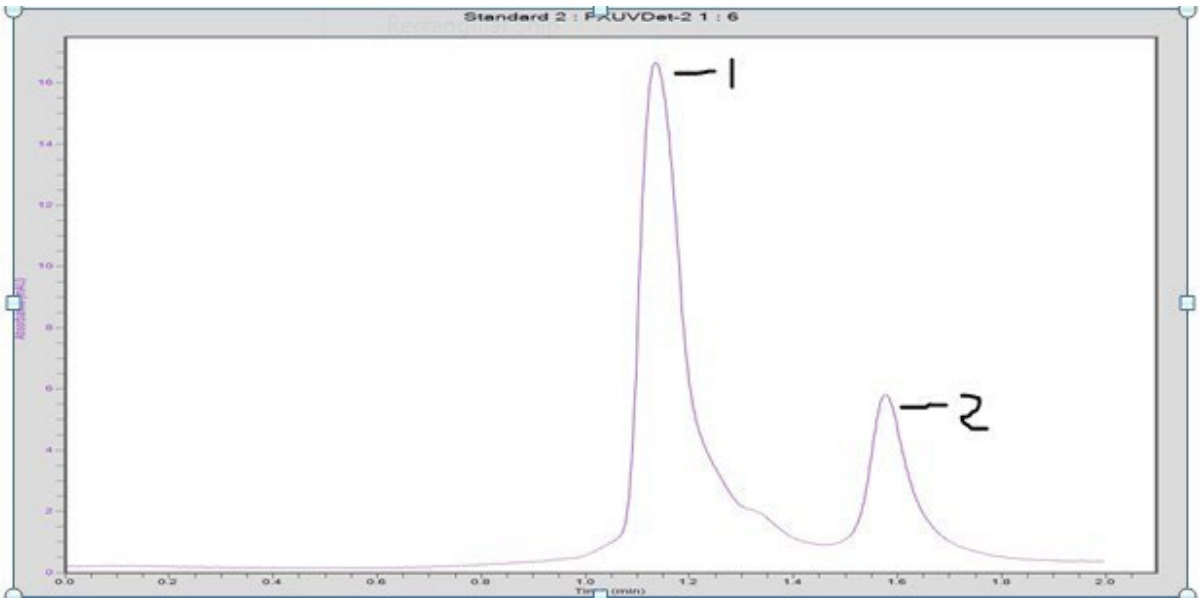


Figure 5.14: HPLC chromatogram. 1: Sotalol (Rt = 1.2 min) (3 ng/mL) and 2: Haloperidol (Rt= 1.79 min) in volunteer plasma, MP (Methanol: Acetonitrile; 50:50, v/v)

5.6.2 Precision of Haloperidol

Precision of haloperidol in plasma was assessed by analyzing three different concentration levels in triplicate namely 10, 50 and 150 ng/ mL respectively. Table 5.7 illustrates the mean and the percentage relative standard deviation (%RSD) data of haloperidol/ sotalol in plasma.

Table 5.7: Precision of haloperidol in volunteer plasma runs in triplicates (n=3)

Concentration (ng/mL)	Mean Peak Area of Haloperidol	%RSD
10	4802.48±311.54	6.49
50	11406.69±313.65	2.75
150	17989.06±240.17	1.34

5.6.3 Accuracy of haloperidol in volunteer plasma

Accuracy refers to the closeness of agreement between the true value of the analyte concentration and the mean result obtained by spiked blank samples. The method recovery was obtained after spiking the additional standard drug solution to the previously analyzed test solution. The value of percentage recovery, % RSDs and SD of haloperidol obtained in plasma was then compared to the values obtained in the mobile phase indicating the accuracy of the proposed method (Table 5.8).

Table 5.8: Accuracy of haloperidol in volunteer plasma

Number of Replicates	Concentration ng/mL		
	3	**150**	**200**
	Area under the Curve (Haloperidol in MP) : Mean ± SD		
N=3	30786.40 ± 1154.4	54116.30 ± 140.0	72019.30 ± 560.6
	Area under the curve (Haloperidol in plasma) : Mean ± SD		
N=3	36536.10 ± 1152.5	57012.70 ± 3874.7	63300.4 ± 3870.0
Overall Mean ± SD	33661.23 ± 3313.91	55564.52 ± 2920.60	67659.83 ± 5377.94
%RSD	9.84	5.26	7.95
%Recovery	118.7%	105.4%	87.9%

MP=Mobile phase; SD=Standard Deviation; %RSD Percentage Relative Standard Deviation

Summary note:

- The variation in the values in the lower concentration range (3 ng/ ml), the % recovery values obtained when the blank plasma was spiked with 3 ng/ ml, were high. This resulted in overestimation of the recovery.

5.6.4 Robustness

To determine the robustness of the method the experimental conditions were altered slightly. Conditions varied as follows: The flow rate of the mobile phase varied from 0.95, 1.0 and 1.1 ml, column oven temperature (20.0 ± 0.5℃), mobile phase composition (51: 49, 50: 50 and 49: 51; buffer: organic solvent mixture v/v). In each case, the %RSD values were calculated for the obtained peak area and retention time. The results obtained were then compared to the results under the normal optimized conditions. The study was performed on the same day and three different days for three different concentrations of haloperidol (six injections). The area obtained from each concentration was compared with that obtained under optimized conditions. The relative standard deviation values were evaluated for each concentration. Table 5.9 below showed variation of haloperidol at different concentrations; 3, 15 and 150 ng/ ml

5.6.4.1 Temperature variation (°C)

The column temperature variation had the potential of affecting the retention time of haloperidol

(Table 5.9).

Table 5.9: Temperature variation of haloperidol at three concentrations (n =3).

(n=3)Tempera-tures (°C)	Mean Retention Time (min)	Mean Peak area	Mean Retention Time (min)	Mean Peak area	Mean Retention Time (min)	Mean Peak area
	3 µg/mL		15 µg/mL		150 µg/mL	
19.5	1.88	5346.46	1.78	7509.33	1.75	30130.8
20	1.72	3429.6	1.71	13064.3	1.71	24318.5
20.5	1.7	5710.83	1.7	10397.9	1.71	29619.1
Overall Mean	1.77	4828.97	1.73	10323.8	1.72	28022.8
SD	0.09	1115.66	0.04	5129.04	0.02	3205.39
%RSD	5.08	23.1	2.31	43.17	1.16	11.4

Summary note:

- From the three tables (Table 5.7, 5.8 and 5.9) above it can be seen that the effect of a change in the temperature causes only a slight variation in the retention time of the compound.
- Lower temperature prolonged the retention time by a small fraction i.e. the higher the temperature the shorter the retention time.
- The variation in the AUC results from change in temperature, hence the large %RSDs.

5.6.4.2 Flow rate variation

A higher or lower flow rate could have adversely affected the quality of chromatography as was shown in Table 5.10.

Summary note:

- From the three tables (Table 5.8, 5.9 and 5.10), it can be seen that the lower the flow rate, the longer the retention time and vice versa.
- Slight variation in the areas were noted however with the flow rate changes, hence the slight large %RSD.

Table 5.10: Flow rate variation of haloperidol tested at three concentrations (n=3).

Flow rate (mL/min)	Mean Retention Time (min)	Mean Peak area	Mean Retention Time (min)	Mean Peak area	Mean Retention Time (min)	Mean Peak area
	3 µg/mL		15 µg/mL		150 µg/mL	
0.95	1.82	14191.8	1.78*	21921.1	1.71	34472.3
1	1.66	18172.3	1.74	26156.6	1.65	39673.9
1.1	1.6*	10120.7	1.61	28704.2	1.61	36256.1
Overall Mean	1.69	14161.6	1.71	25594	1.66	36800.7
SD	0.11	3266.1	0.08	5787.91	0.04	2894.27
%RSD	6.51	25.55	4.68	20.99	2.41	7.86

*: The highlighted value was skewing the data, thus was not taken into account in the calculation.

5.6.4.3 Mobile phase variation (in percentage %)

A slight variation in the organic portion of the mobile phase has the effect of either increasing or decreasing the retention times as shown in the Table 5.11.

Table 5.11 Mobile phase was varied to optimize haloperidol detection (n=3), where the mobile phase was an organic buffer (Methanol: Acetonitrile; 50/50 v/v).

Mobile phase (%)	Mean Retention Time (min)	Mean Peak area	Mean Retention Time (min)	Mean Peak area	Mean Retention Time (min)	Mean Peak area
	3 µg/mL		15 µg/mL		150 µg/mL	
49:51:00	1.74	8000.45	1.74	13919	1.74	31108.5
50:50:00	1.75	7705.98	1.75	16687.7	1.75	31725.2
51:49:00	1.76	10416.2	1.76	19033.2	1.76	32212.5
Overall Mean	1.75	8707.54	1.75	16546.6	1.75	31682.1
SD	0.009	1925.58	0.009	2423.61	0.009	669.25
%RSD	0.51	22.11	0.51	14.65	0.51	2.11

Summary note:

- Slight change in the organic component of the mobile phase also had an effect on the retention i.e. the higher the organic mobile phase the shorter the retention and vice versa.
- Slight variation in the AUC were noted however with the mobile phase variation, hence the slightly large %RSDs.

5.7 Tramadol in plasma

5.7.1 Linearity

Table 5.12 below shows the linearity equation of the standard curve, LOD and LOQ including other relevant parameters. The regression curve showed that the compound was linear between the ranges of 1-200 ng/ ml (Figure 5.15).

Table 5.12: Linearity and regression parameters of tramadol with precision values

Parameters	Values
Linear range, ng mL^{-1}	1-200
Limit of detection (LOD), ng mL^{-1}	0.004
Limit of quantification (LOQ), ng mL^{-1}	0.01
Regression equation	Y = mX + c
Correlation coefficient (R^2)	0.9934

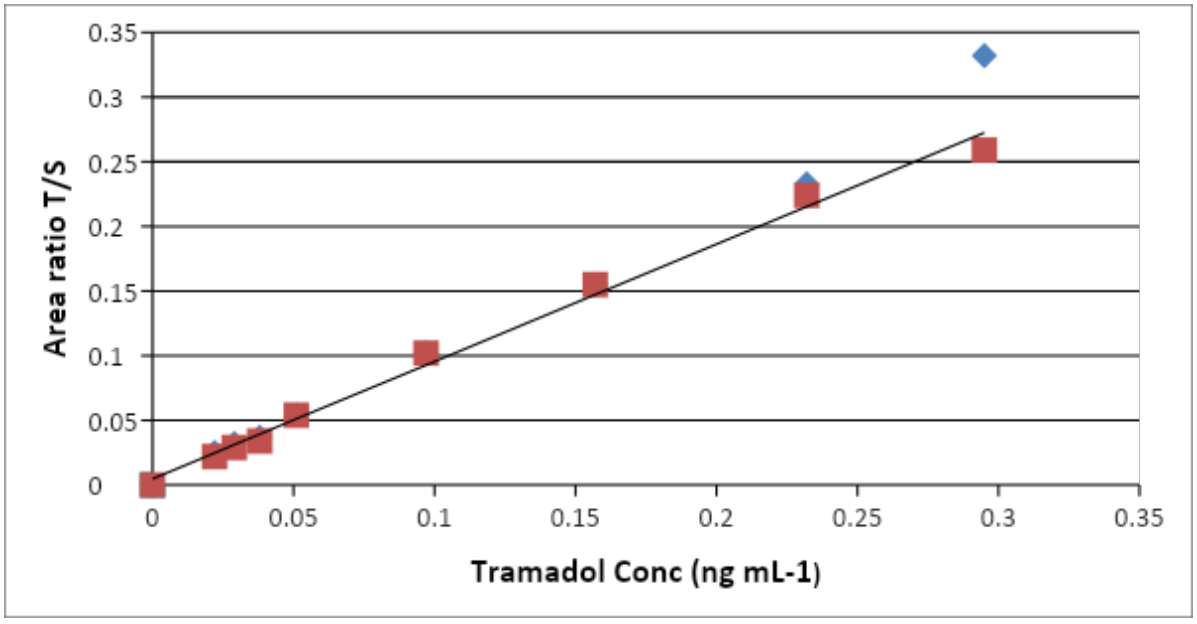


Figure 5.15: Calibration curve of Tramadol in volunteer/ blank plasma with the mobile phase consisting of Methanol: Acetonitrile, 50:50, (v/v).

Summary note:

- The standard curve showed linearity of Tramadol in plasma (Figure 5.16)
- The compound is well separated with the internal standard sotalol in volunteer/ blank plasma (Figure 5.16).

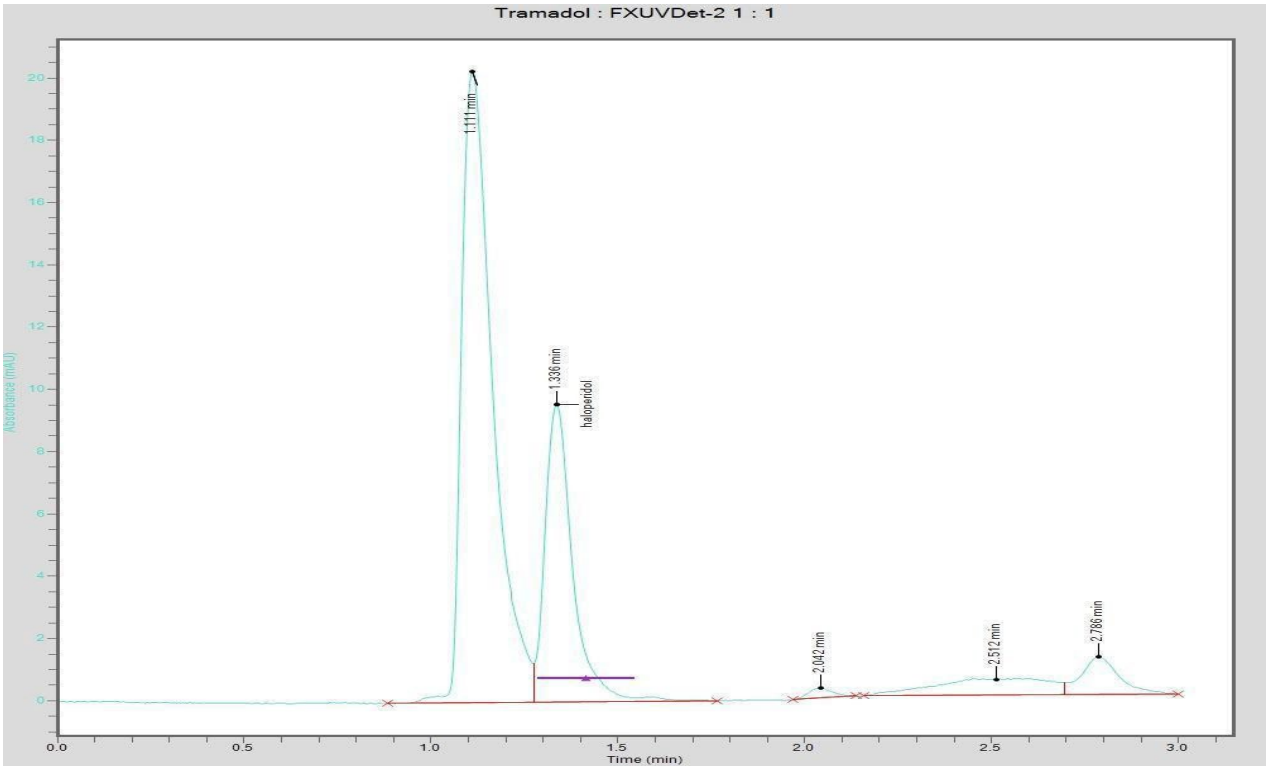


Figure 5.16: HPLC chromatogram of Tramadol/ Sotalol (3 ng/ ml) in volunteer/blank plasma. Sotalol (Rt= 1.2 min), Tramadol (Rt= 1.4 min).

5.7.2 Precision

The reproducibility and intermediate precision of Tramadol in plasma are shown in Table 5.13.

Summary note:

- The results of repeatability (intraday precision) and intermediate (interday precision) were all reported in terms of %RSD. The results of which were all below 2% which is in line with acceptable criteria.
- This demonstrated the Tramadol stability and the proposed method reliability.

Table 5.13: Precision of proposed method of Tramadol in volunteer/ blank plasma over (over 3 days)

Concen-tration	Peak Area of tramadol	Intraday precision		Days	Interday precision		
		SD	%RSD		Peak Area of tramadol	SD	%RSD
	32627.08			1	27943.62		
100	33006.81			2	27802.59		
	33076.08			3	29381.76		
Mean	**32903.32**	**241.73**	**0.73**		**28375.99**	**873.87**	**3.08**
	42883.86			1	49215.03		
150	42878.18			2	48840.38		
	42705.48			3	49806.64		
Mean	**42822.51**	**101.39**	**0.24**		**49287.35**	**487.17**	**0.1**
	43607.69			1	52698.19		
200	43465.41			2	54570.97		
	43137.25			3	53254.57		
Mean	**43403.45**	**241.26**	**0.56**		**53507.91**	**961.75**	**1.8**

5.7.3 Accuracy and % recovery of Tramadol

The proposed method accuracy was obtained after spiking the specific standard drug solution to the previously analyzed test solution. The value of percentage (%) recovery, SD and % RSD are shown in Table 5.14.

Summary note:

- The results above indicate the accuracy of the proposed method in volunteer/blank plasma
- It was observed that at the 200 ng/ ml concentration of tramadol indicated an above average percentage recovered. It could be due to a variation in peak integration.
- Low concentrations of tramadol indicated an under-recovery of tramadol. This could be due to the difficulty of the integration of the peaks by the software.

Table 5.14: Accuracy and recovery of Tramadol in volunteer/blank plasma of three replicates.

Number of Replicates	Concentration Spiked ng/mL		
	3	**150**	**200**
	Area under the Curve (TRM in MP)		
1	16965.80	227721.50	269491.90
2	14824.20	227893.40	268938.00
3	15634.60	229789.60	260018.90
Mean	**15808.20**	**228468.17**	**266149.60**
	Area under the curve (TRM in plasma)		
1	11488.80	286517.50	333405.60
2	11846.10	290325.60	392764.60
3	10300.30	285124.70	393890.90
Mean	**11211.73**	**287322.60**	**373353.70**
Overall Mean	**13509.97**	**257895.38**	**319751.65**
SD	2658.56	32288.99	62753.52
%RSD	23.71	11.24	16.81
%Recovery	70.92	125.76	140.28

5.7.4 Robustness

The robustness of Tramadol in volunteer/ blank plasma was tested by changing some analysis conditions. This proposed method measured the robustness by means of altering quantitative factors such as temperature, flow rate and mobile phase.

5.7.4.1 Temperature Variation (°C)

High temperature help accelerate particle movement however, high temperatures in the chromatographic process lead to shortened retention times as shown in Table 5.15-5.17.

Table 5.15: Temperature variation of Tramadol (3 µg/ ml),

Temperatures (°C)	Number of Replicates	Retention Time (min) Mean	Peak area Mean
19.5	N=3	1.40	6495.02
20.0	N=3	1.40	11807.09
20.5	N=3	1.36	8298.17
Overall Mean		1.39	8866.76
SD		0.02	2485.08
%RSD		1.44	28.03

Table 5.16: Temperature variation of Tramadol (15 µg/ ml),

Temperatures (°C)	Number of Replicates	Retention Time (min) Mean	Peak area Mean
19.5	N=3	1.43	12995.86
20.0	N=3	1.42	15095.22
20.5	N=3	1.38	16687.67
Overall Mean		1.41	14926.25
SD		0.02	3031.34
%RSD		1.42	19.48

Summary note:

- A slight change in temperature varied the retention time slightly i.e. a higher temperature causes the Rt to decrease and vice versa
- Slight variation in the areas were noted however, with the temperature changes, hence the larger %RSDs

Table 5.17: Temperature variation of Tramadol (150 µg/ ml).

Temperatures (°C)	Number of Replicates	Retention Time (min) Mean	Peak area Mean
19.5	N=3	1.35	222277.94
20.0	N=3	1.34	222277.94
20.5	N=3	1.32	322125.85
Overall Mean		1.34	255560.58
SD		0.02	51717.47
%RSD		1.49	20.24

5.7.4.2 Flow rate variation (ml/ min)

Flow rate plays an important role in the HPLC. The solvent moves the sample through the system to reach the stationary phase where separation and identification of each component occurs. The flow rate was adjusted as follows: 0.95, 1.0 and 1.1 ml/ min (Table 5.18-5.20)

Table 5.18: Flow rate variation of Tramadol (3 µg/ ml)

Flow Rate (ml/ min)	Number of Replicates	Retention Time (min) Mean	Peak area Mean
0.95	N=3	1.39	12230.26
1.0	N=3	1.37	15808.17
1.1	N=3	1.37	10805.23
Overall Mean		1.38	12947.89
SD		0.01	2379.24
%RSD		0.72	18.38

Table 5.19: Flow rate variation of Tramadol (15 µg/ ml)

Flow Rate (ml/ min)	Number of Replicates	Retention Time (min) Mean	Peak area Mean
0.95	N=3	1.38	21805.84
1.0	N=3	1.37	21235.67
1.1	N=3	1.37	21769.84
Overall Mean		1.37	21603.78
SD		0.005	370.22
%RSD		0.36	1.71

Table 5.20: Retention time variation of Tramadol (150 µg/ ml)

Flow Rate (ml/ min)	Number of Replicates	Retention Time (min) Mean	Peak area Mean
0.95	N=3	1.38	244327.20
1.0	N=3	1.35	228468.18
1.1	N=3	1.34	244415.20
Overall Mean		1.36	239070.19
SD		0.18	7972.93
%RSD		2.45	3.33

Summary note:

- Change in flow rate resulted in variation in Rt, with a higher flow rate resulting in a shorter Rt and vice versa.
- Areas remained relatively similar with the variation in flow rate
- %RSD varies according to the concentrations.

5.7.4.3 Mobile phase variation (%)

Table 5.21 showed mobile phase variation at different concentrations (3, 15 and 150 ng/ ml). The HPLC mobile phase chromatographic condition of Tramadol was 50% acetonitrile and 50% phosphate buffer at a pH of 2.8. The ratio of the organic component of the mobile phase was varied by ±1%. Across the different concentrations and through the mobile phase adjustment, the average retention time was reported.

Table 5.21: Mobile phase variations used to optimize Tramadol detection.

Mobile phase (%) Organic phase buffer	Number of Replicates	Mean Retention Time (min)	Mean Peak area	Mean Retention Time (min)	Mean Peak area	Mean Retention Time (min)	Mean Peak area
		3 µg/mL		15 µg/mL		150 µg/mL	
49:51:00	N=3	1.36	16931.4	1.35	23873.2	1.35	359410.6
50:50:00	N=3	1.34	19073.9	1.34	21235.7	1.34	319403.9
51:49:00	N=3	1.26	15664.2	1.31	24549.0	1.31	325582.0
Overall Mean		1.32	17223.2	1.33	23219.3	1.33	334798.8
SD		0.05	1638.74	0.02	2159.75	0.02	19433.9
%RSD		3.79	9.51	1.5	9.3	1.5	5.8

Summary note:

- Despite the change in mobile phase chromatographic conditions the peak area of the concentrations remains relatively constant.
- Slight change in the organic component of the mobile phase also has an effect on the retention i.e. the higher the organic mobile phase the shorter the retention and vice versa.
- %RSD varies according to the concentrations hence the values were above 2%

5.7.5 Solution stability and mobile phase stability

Stability of Haloperidol and Tramadol solutions was studied by injecting the sample into the chromatographic system at different time intervals (24 hour interval). The peak area was recorded in the time intervals of samples stored at -20°C and compared to samples stored at 4°C temperature. The %RSD values of the peak areas were calculated and presented in Table 5.22 and 5.23.

Table 5.22 Stability: Haloperidol in mobile phase and volunteer/ blank plasma stored at different temperatures.

Concentration (ng/mL)	Mobile phase (4°C) Mean Area ± SD	Extracted plasma (-20°C) Mean Area ± SD	Overall Mean Area ± SD
3	32558.63 ± 143.99	26971.05 ± 633.40	29764.84 ± 388.70
%RSD	**0.44**	**2.35**	**1.31**
15	59988.15 ± 184.40	43361.50 ± 1816.14	51674.83 ± 1000.27
%RSD	**0.31**	**4.19**	**1.94**
150	628098.82 ± 534.43	550592.42 ± 855.78	589345.62 ± 695.11
%RSD	**0.09**	**0.16**	**0.12**

Table 5.23 Stability: Tramadol in mobile phase and volunteer/ blank plasma stored at different temperatures

Concentration (ng/ ml)	Mobile phase (4°C) Mean Area ± SD	Extracted plasma (-20°C) Mean Area ± SD	Overall Mean Area ± SD
3	17078.67 ± 648.30	16308.11 ± 285.71	16693.39 ± 1767.01
%RSD	3.80	17.70	10.60
15	62887.32 ± 4009.99	32558.63 ± 143.99	47722.98 ± 2076.99
%RSD	6.38	0.44	4.35
150	359410.63 ± 10793.84	303940.36 ± 2289.96	331675.50 ± 6541.90
%RSD	3.00	0.75	1.97

Summary note:

- Haloperidol stability in plasma showed slight degradation (17.1, 27.7, 6.2% at 3, 15 and 150 ng/ ml) respectively.
- Tramadol stability in plasma also showed degradation (4.5, 48.2 and 15.4% at 3, 15 and 150 ng/ ml respectively.
- %RSD varied according to the concentrations.

5.7.6 Haloperidol Concentrations in Patients' Plasma

The concentration of haloperidol in patients' plasma was assessed by using the slope of the standard curve for peak-area ratios for the analytes and internal standard (Table 5.32).

Table 5.24 Haloperidol concentrations (ng/ ml) in delirium ICU patients.

Patients Number	Area 1 HLP/SOT	Area 2 HLP/SOT	Area 3 HLP/SOT	Overall Mean	Overall SD	Overall %RSD
1.	0.0090	0.0090	0.0080	0.0087	0.0006	6.67
HLP Conc (ng/mL)	48.06	48.06	41.90	46.01	3.56	7.73
2.	0.0080	0.0090	0.0090	0.0087	0.0006	6.66
HLP Conc (ng/mL)	41.90	48.06	48.06	46.01	3.56	7.73
3.	0.0120	0.0110	0.0190	0.0140	0.0044	31.13
HLP Conc (ng/mL)	66.55	60.39	60.39	62.44	3.56	5.70
4.	0.0040	0.0030	0.0040	0.0037	0.0006	15.75
HLP Conc (ng/mL)	17.25	11.092	17.25	15.20	3.56	23.39
5.	0.0034	0.0028	0.0032	0.0031	0.0003	9.75
HLP Conc (ng/mL)	13.56	9.86	12.32	11.91	1.88	15.8
6.	0.0030	0.0020	0.0032	0.0027	0.0006	23.52
HLP Conc (ng/mL)	11.09	4.93	11.09	9.04	3.56	39.37
7.	0.0089	0.0060	0.0070	0.0073	0.0015	20.18
HLP Conc (ng/mL)	45.60	29.60	35.74	36.97	8.08	21.86
8.	0.0030	0.0030	0.0020	0.0027	0.0006	21.65
HLP Conc (ng/mL)	11.09	11.09	4.93	9.04	3.56	39.37
9.	0.0110	0.0100	0.0090	0.010	0.0010	10.00
HLP Conc (ng/mL)	60.39	54.23	48.06	54.23	6.16	11.36
10.	0.0038	0.0038	0.0035	0.0037	0.0002	4.68
HLP Conc (ng/mL)	16.02	16.02	14.17	15.41	1.07	6.93
11.	0.0032	0.0032	0.0031	0.0032	0.0001	1.82
HLP Conc (ng/mL)	12.32	12.32	11.71	12.12	0.36	2.93
12.	0.0048	0.0048	0.0047	0.0048	0.0001	1.21
HLP Conc (ng/mL)	22.18	22.18	21.57	21.98	0.36	1.62
13.	0.0041	0.0043	0.0034	0.0039	0.0005	11.80

HLP Conc (ng/mL)	18.06	18.56	13.56	16.72	2.75	16.47
14.	0.0050	0.0070	0.0070	0.0063	0.0012	18.23
HLP Conc (ng/mL)	23.42	35.74	35.74	31.63	7.12	22.50
15.	0.0033	0.0030	0.0029	0.0031	0.0002	7.06
HLP Conc (ng/mL)	12.94	10.85	10.48	11.42	1.33	11.64
16.	0.0071	0.0069	0.0071	0.0070	0.0001	1.64
HLP Conc (ng/mL)	36.36	35.12	36.36	35.95	0.71	1.98
17.	0.0040	0.0041	0.0035	0.0039	0.0003	8.31
HLP Conc (ng/mL)	17.25	17.87	14.17	16.43	1.98	12.05
18.	0.0132	0.0132	0.0013	0.0092	0.0068	74.5
HLP Conc (ng/mL)	73.94	73.94	72.10	73.33	1.07	1.46
19.	0.0018	0.0020	0.0020	0.0020	0.0001	6.82
HLP Conc (ng/mL)	3.70	5.18	5.05	4.64	0.82	17.68
20.	0.0016	0.0014	0.0013	0.0014	0.0002	10.66
HLP Conc (ng/mL)	2.47	1.23	0.62	1.44	0.94	65.49

5.7.7 Haloperidol and Tramadol determination in patient's urine

5.7.7.1 Haloperidol determination in mobile phase

5.7.7.1.1 Linearity (n=3)

Haloperidol eluted at 1.70 min (Figure 5.18) and a haloperidol standard curve in mobile phase was constructed (Figure 5.19).

The standard curve and other parameters are presented in Section 5.1 and 5.3, respectively.

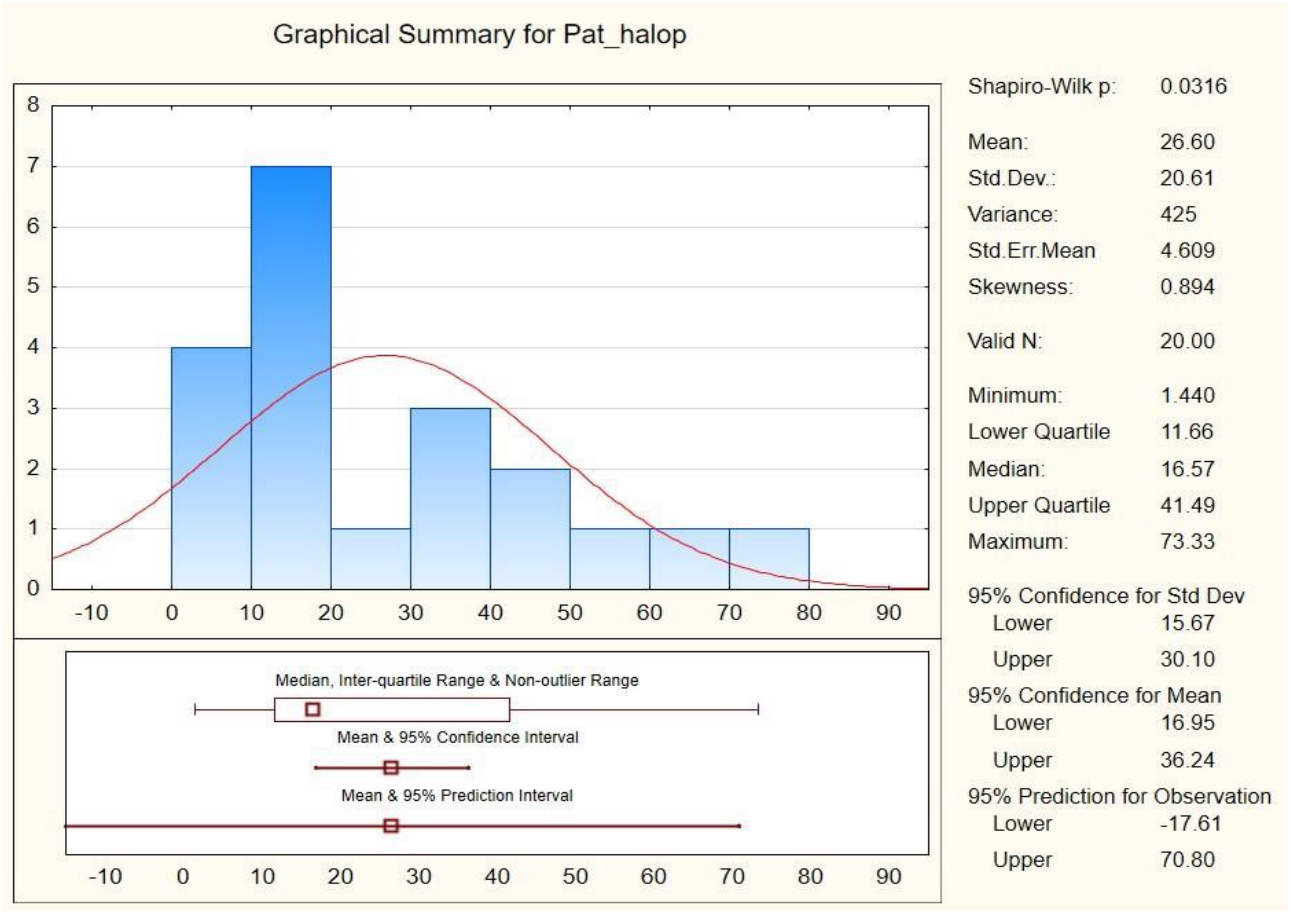


Figure 5.16: Normality distribution and variables of ICU patients' on haloperidol.

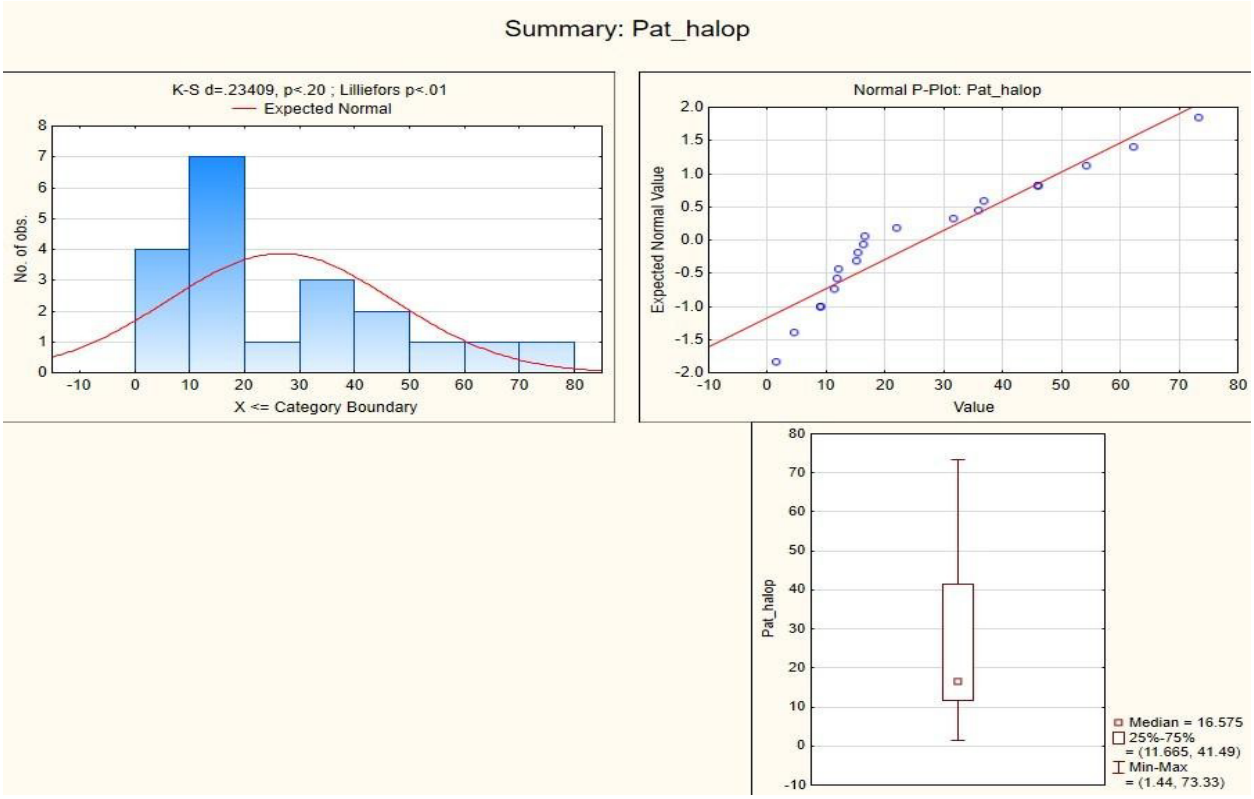


Figure 5.17: Summary of ICU patients' on haloperidol. Where, Lowest 1.44 ± 0.94 ng/ml, Highest 73.33 ± 1.07 ng/ml, Range: 1.44 ± 0.94 to 73.33 ± 1.07 ng/ml, Normal plasma range for patients on Haloperidol: 5 – 17 ng/ml

5.7.8 Haloperidol determination in volunteer urine

5.7.8.1 Urine accuracy

Healthy volunteer urine samples were found to contain a large peak that eluted at 1.1 min, followed by a smaller peak at 1.4 min (Figure 5.20). Spiked haloperidol was quantifiable in healthy volunteer urine (Figure 5.21) as the haloperidol peak eluted at 1.7 min. However, it was difficult to accurately quantify the haloperidol in patients' urine. There is a little or no presence of drug detected in all the patient's urine samples tested for haloperidol. However, healthy volunteer urine was spiked with haloperidol in order to determine the accuracy of the compound compared to detection in the mobile phase.

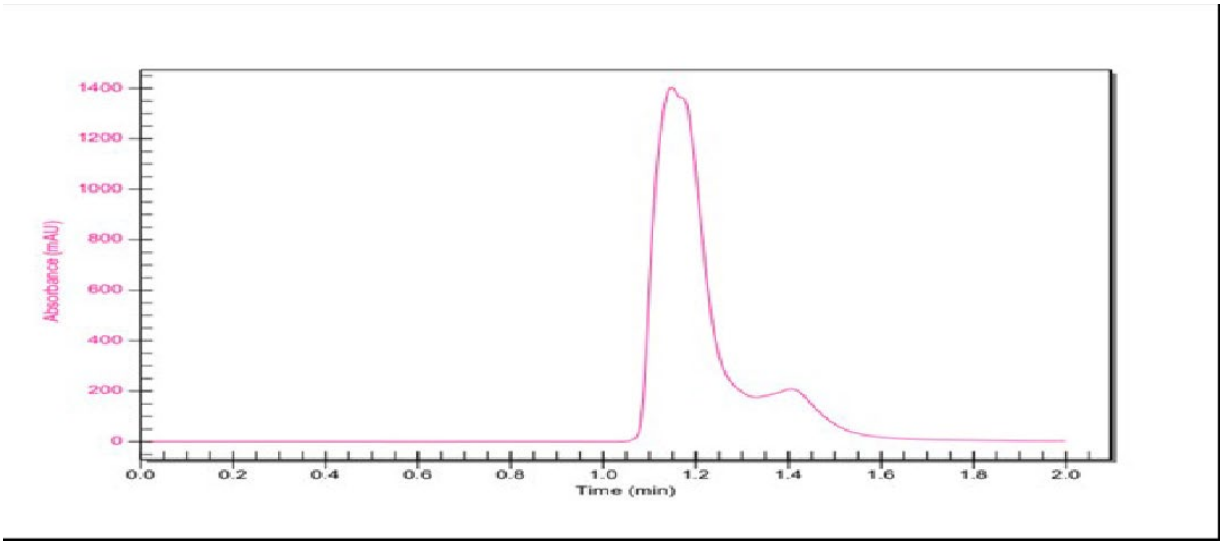


Figure 5.20: Healthy volunteer urine chromatogram

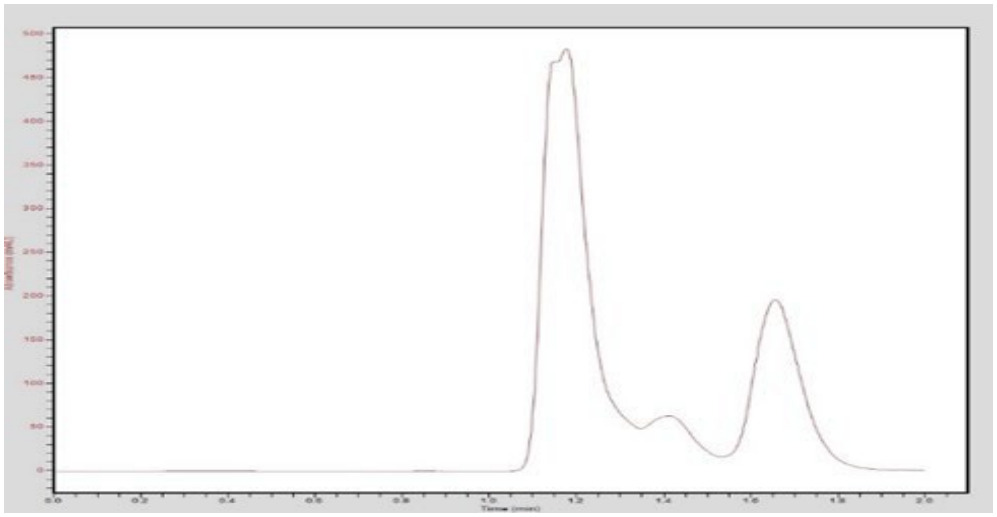


Figure 5.21: Haloperidol-spiked volunteer urine (50 µg/ ml RT- 1.7 min)

Accuracy results obtained by comparing the haloperidol-spiked urine samples (1, 20 and 50 µg/ml) to the same samples in mobile phase are presented in Table 5.34.

Table 5.25: Accuracy of haloperidol-spiked volunteer urine compared to haloperidol in mobile phase. Each concentration was done in triplicate.

Concentration Post-Spiking ng/ml	Mean peak area (n=3 injections)	Amount determined (ng/ml)	% Recovery
1	not detected	0	0
20	680403 ± 292	18.7 ± 0.0	94.0 ± 0.0
50	1499312 ± 6742	41.6 ± 0.2	83.0 ± 0.0

5.7.9 Patients' urine samples

Each patient sample was analyzed using the urine precipitation method stated in section 2.2. The results are presented in the chromatograms (Figure 5.22). Patient A001 displayed no sign of haloperidol in the urine and lacked the component that appears at Rt 1.4 min in healthy urine.

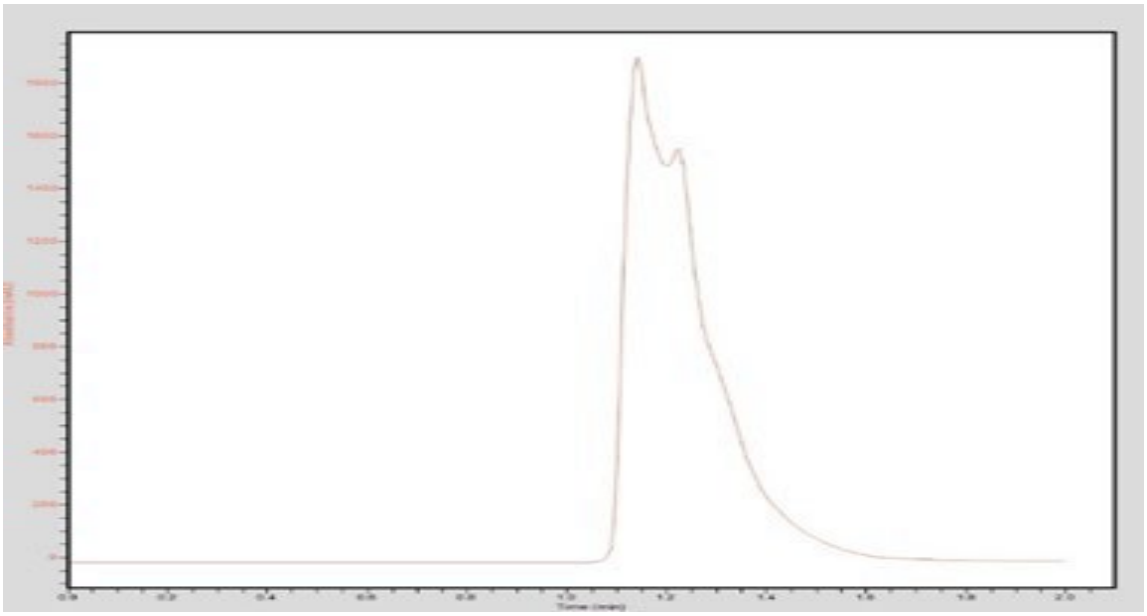


Figure 5.22: Urine sample of patient A001

Patient A006 and A067's urine had a higher amount of the initial peak (maybe urea) and displayed no sign of haloperidol in the urine. The patients also lacked the component that appears at RT 1.4 min in healthy urine (Figure 5.23 and 5.24).

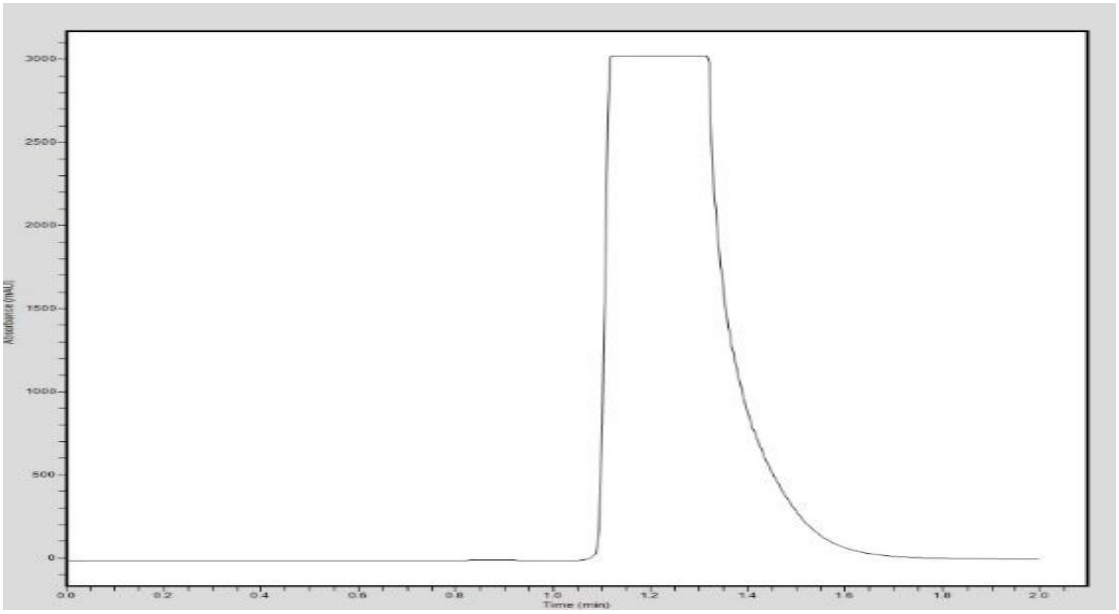


Figure 5.23: Urine samples of patients A006 and A067

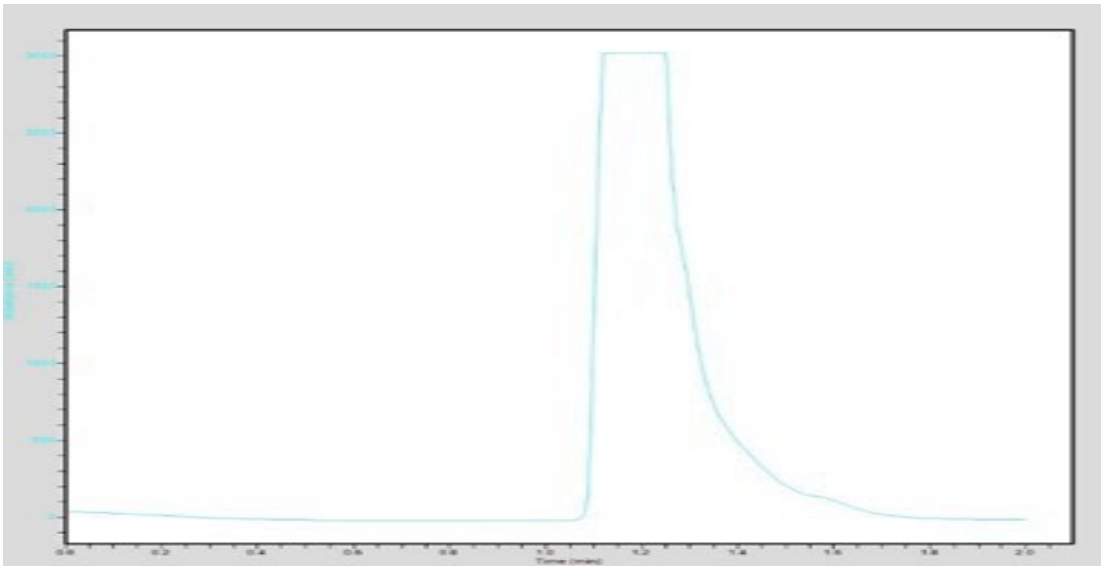


Figure 5.24: Urine samples of patient A067

The urine of patients A070 and A076 also had a peak similar to that found in healthy volunteer urine with a peak indicating the presence of urea and/or creatinine however, indicated no presence of haloperidol in the urine (Figure 5.25). This may largely be due to the fact that most of our patients

were managed as a case of acute kidney injury which may partly explain why the level was too low to be detected. Another explanation may be as a result of long-standing storage of the urine samples for over 3 years; hence causing the samples to lose their stability. Finally, some drugs were reported to reduce the plasma levels of haloperidol, such as anti-tuberculosis, antifungal as well as barbiturates in addition to anti-epileptic drugs. Moreover, all of these medications were used at some point in the course of treatment of patients.

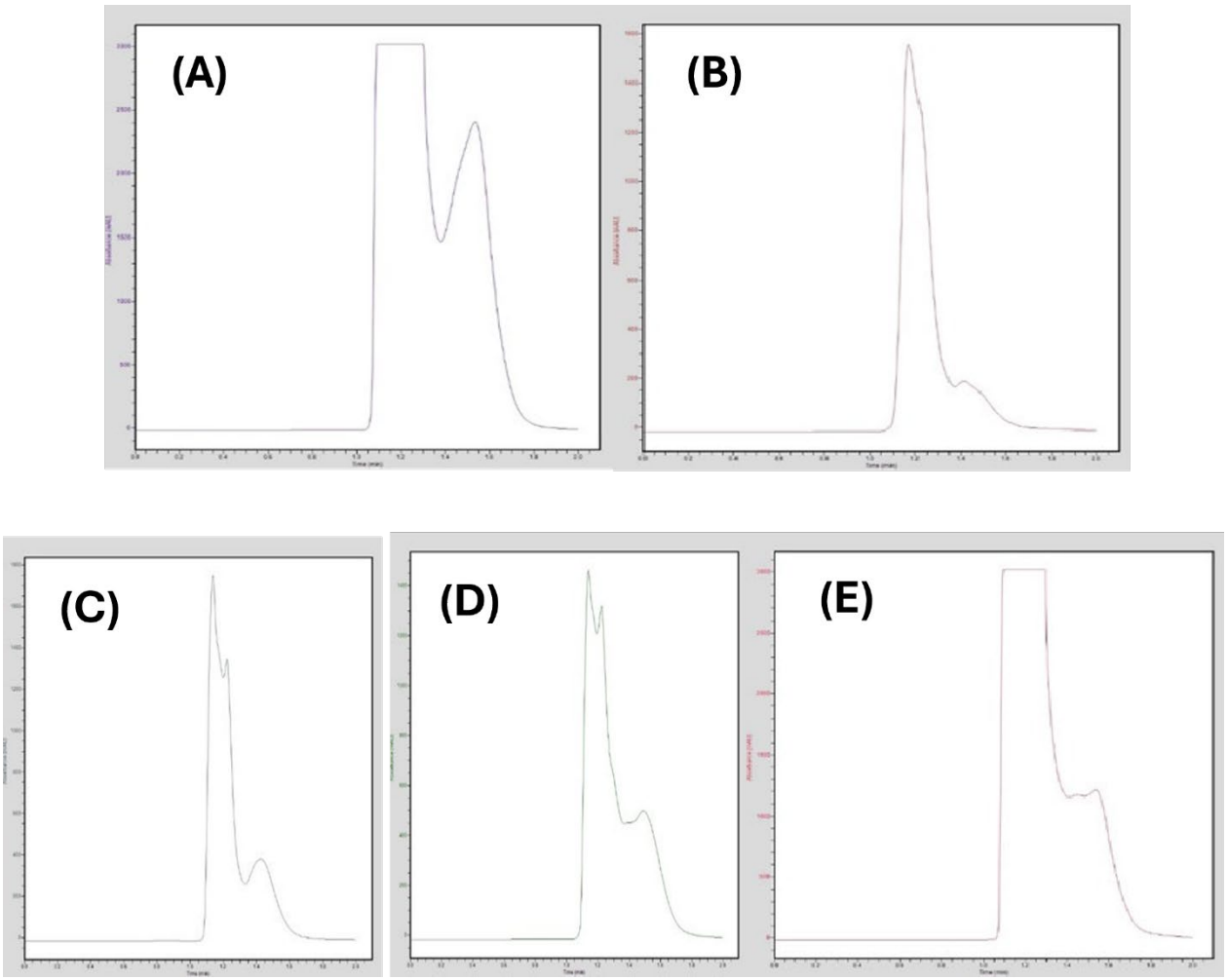


Figure 5.25: Urine samples of patients (A) A070 and (B) A076 compared to (C) A012, (D) A029 and (E) A034

5.7.10 Research Limitations and Future Recommendations

The method was successfully developed for the detection of haloperidol in the mobile phase. However, due to the presence of urea and/or creatinine in urine that elutes very closely with haloperidol, the method is not suitable for the detection of haloperidol in urine. It is suggested that a method that elutes haloperidol at least 0.4 minutes away from the urine contents/ eluents be validated.

The following methods were tested for future perspectives.

At a mobile phase ratio 40:60 acetonitrile: buffer compared to the initial conditions, haloperidol eluted with a Rt of 2.4 min from haloperidol-spiked volunteer urine (Figure 5.26 final concentration 25 µg/ ml).

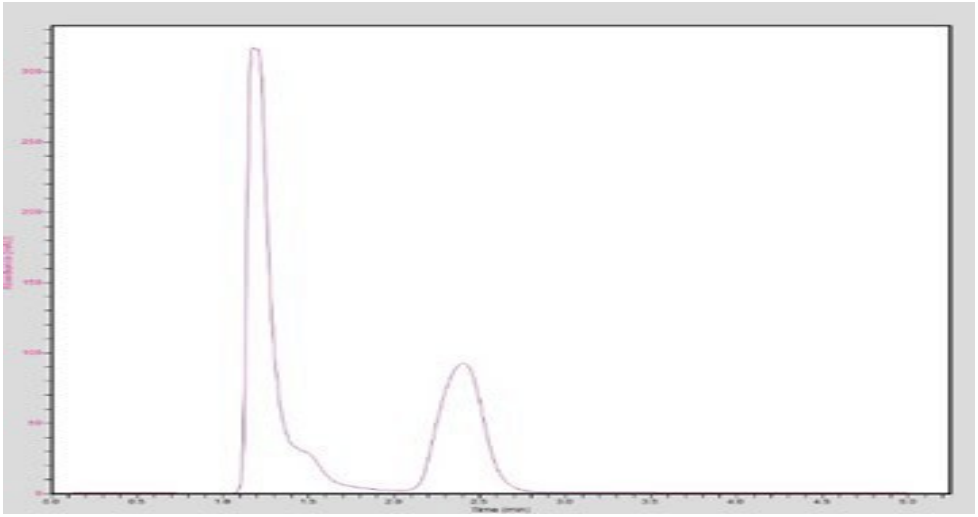


Figure 5.26: Chromatogram showing Haloperidol in urine (25 µg/ ml) with a RT of 2.4 min. Chromatographic conditions: 0.05M potassium phosphate buffer (pH 2.8): 0.05 M potassium phosphate buffer: acetonitrile (60:40 v/v), FR: 1 ml/ min, run time of 5min, wavelength 240 nm.

5.8 Tramadol Concentration in Patients' Plasma

Peak heights in the chromatograms were measured manually. Concentration tramadol were assessed by using the slope of the standard curve for peak-height ratios for the analytes and internal standard as shown in Table 5.26 and Figures 5.18.

Table 5.26 Tramadol concentration in delirium ICU patients

No of patients	Area 1 TRM/SOT	Area 2 TRM/SOT	Area 3 TRM/SOT	Overall Mean	Overall SD	Overall %RSD
1.	0.174	0.192	0.184	0.183	0.009	4.919
TRM Conc (ng/mL)	16.315	18.052	17.280	17.216	0.870	5.055
2.	0.149	0.149	0.153	0.150	0.002	1.536
TRM Conc (ng/mL)	13.903	13.903	14.289	14.032	0.223	1.588
3.	0.152	0.149	0.133	0.145	0.010	7.061
TRM Conc (ng/mL)	14.193	13.903	12.359	13.485	0.986	7.311
4.	0.577	0.610	0.537	0.575	0.037	6.361
TRM Conc (ng/mL)	55.198	58.382	51.338	54.973	3.527	6.417
5.	0.109	0.104	0.103	0.105	0.003	3.052
TRM Conc (ng/mL)	10.043	9.561	9.465	9.689	0.309	3.197
6.	0.269	0.289	0.249	0.269	0.02	7.435
TRM Conc (ng/mL)	25.481	27.439	23.551	25.490	1.944	7.626
7.	0.111	0.062	0.029	0.067	0.041	61.276
TRM Conc (ng/mL)	10.237	5.09	2.325	6.024	3.981	66.089
8.	0.675	0.859	0.7	0.744	0.099	13.402
TRM Conc (ng/mL)	58.633	82.406	67.065	69.368	17.375	12.053
9.	0.264	0.268	0.267	0.266	0.002	0.782
TRM Conc (ng/mL)	24.999	25.385	25.288	25.224	0.201	0.796
10.	0.539	0.508	0.521	0.523	0.016	2.978
TRM Conc (ng/mL)	51.531	48.540	49.795	49.955	1.502	3.007
11.	0.157	0.147	0.142	0.149	0.008	5.137
TRM Conc (ng/mL)	14.675	13.710	13.228	13.871	0.737	5.321
12.	0.149	0.135	0.144	0.143	0.007	4.973
TRM Conc (ng/mL)	13.903	12.552	13.421	13.292	0.685	5.151

13.	0.106	0.102	0.105	0.104	0.002	1.995
TRM Conc (ng/mL)	9.754	9.368	9.658	9.593	0.201	2.095
14.	0.115	0.114	0.116	0.115	0.001	0.869
TRM Conc (ng/mL)	10.623	10.526	10.719	10.623	0.097	0.908
15.	0.138	0.145	0.130	0.138	0.008	5.452
TRM Conc (ng/mL)	12.842	13.517	12.069	12.809	0.725	5.656
16.	0.108	0.113	0.109	0.11	0.003	2.405
TRM Conc (ng/mL)	9.947	10.429	10.044	10.140	0.255	2.514
17.	0.281	0.293	0.297	0.290	0.008	2.868
TRM Conc (ng/mL)	26.639	27.797	28.183	27.539	0.804	2.918
18.	0.393	0.395	0.391	0.393	0.002	0.509
TRM Conc (ng/mL)	37.440	37.638	37.252	37.443	0.193	0.516
19.	0.157	0.123	0.121	0.134	0.020	15.136
TRM Conc (ng/mL)	14.675	11.395	11.202	12.424	1.952	15.710
20.	0.246	0.252	0.225	0.241	0.0142	5.883
TRM Conc (ng/mL)	23.262	23.841	21.236	22.779	1.368	6.005
21.	0.102	0.102	0.104	0.103	0.0011	1.125
TRM Conc (ng/mL)	9.368	9.368	9.561	9.432	0.111	1.181
22.	0.551	0.621	0.664	0.612	0.057	9.319
TRM Conc (ng mL^{1})	52.689	59.443	63.590	58.574	5.502	9.394
23.	0.399	0.286	0.285	0.323	0.066	20.267
TRM Conc (ng/mL)	38.024	27.121	27.025	30.723	6.323	20.579
24.	0.195	0.198	0.192	0.195	0.003	1.538
TRM Conc (ng/mL)	18.341	18.631	18.052	18.341	0.289	1.578

TRM = Tramadol, Conc = Concentration

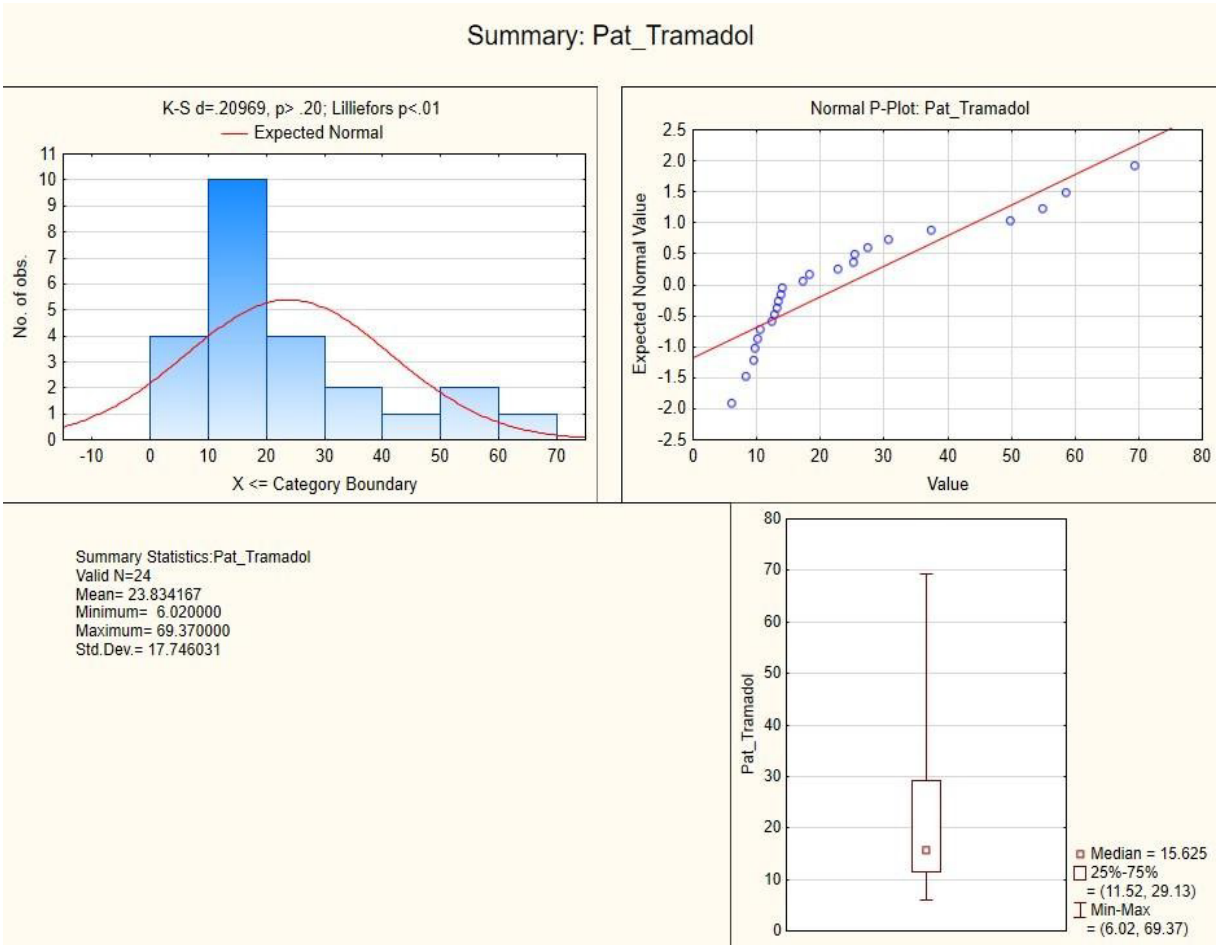


Figure 5.18: Normality distribution of tramadol. Where Lowest 6.02 ± 3.98 ng/ ml; Highest 69.37 ± 17.38 ng/ ml; Range: 6.02 ± 3.98 to 69.37 ± 17.38 ng/ ml; Normal plasma range for patients on Tramadol: 80 – 100 ng/ ml. For practical purposes a bi-sigmoidal model was used for data analysis. The results indicated a significant relationship between tramadol plasma level and therapeutic effect tramadol on the patient management ($p < 0.001$).

5.8.1 Tramadol determination in patients' urine samples

- Tramadol was found to co-elute with a large peak in urine (Rt 1.1 – 1.6 min), possibly urea and creatinine, therefore the quantification was not possible (Figure 5.19).

5.8.2 Research Limitation and Future Recommendations

To increase the Rt of tramadol so that it does not co-elute with the major initial peaks in urine, a MP ratio of 20:80 acetonitrile: buffer, compared to that of the initial conditions were tested. Tramadol eluted with a Rt of 2.4 min in mobile phase (Figure 5.19a, final concentration 100 µg/ ml). However, when volunteer urine was spiked with tramadol at the same concentration, the tramadol

peak expected at 2.4 min disappeared, suggesting a possible interaction between urea and / or creatinine with tramadol as presented in Figure 5.19b below:

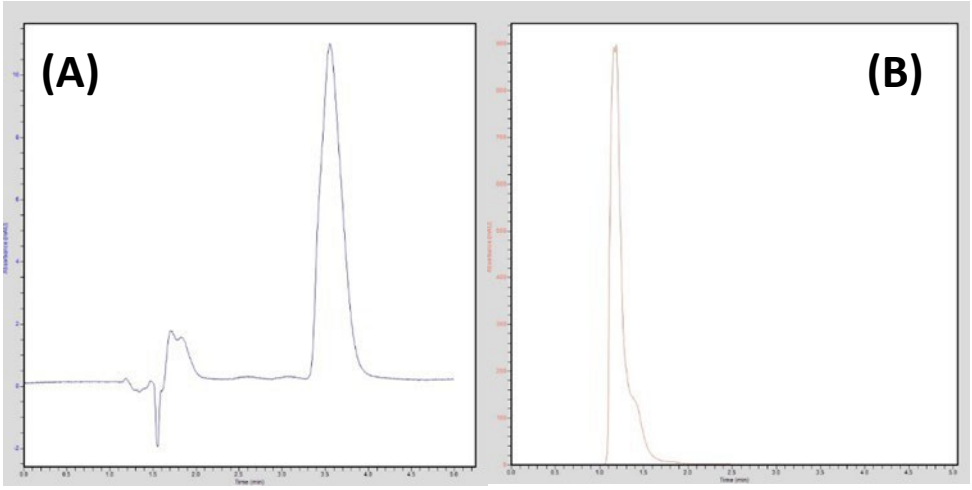


Figure 5.19: Chromatogram showing (a) Tramadol (100 µg/ ml) in mobile phase with a Rt of 2.4 min; (b) Volunteer urine spiked with tramadol at 80:20 mobile phase ratio. Chromatographic conditions: 0.05M potassium phosphate buffer (pH 2.8): 0.05 M potassium phosphate buffer: acetonitrile (80:20 v/v), FR: 1 ml/ min, run time of 5min, wavelength 250 nm.

To prevent interaction with urine contents, a solid phase extraction technique (SPE) is suggested to remove any contaminating urine content before quantifying the drugs themselves. This technique will also concentrate and purify the drugs before detection. Due to the fact that haloperidol is excreted at only 1% and tramadol at 30%, respectively in urine, the drugs would need to be concentrated to improve detectability.

CHAPTER SIX

RESULTS AND DISCUSSION: SPECTROPHOTOMETRY AND ELISA

6.0 Patient Blood Sample Preparation - For both the HPLC and ELISA

Whole blood from consenting patients was drawn into EDTA-containing blood collection tubes during the daily routine ward rounds either in the morning or evening (5 ml). The EDTA-blood tubes were centrifuged at 5000 rpm for 10 min at room temperature. The plasma was aliquoted into micro centrifuge tubes and stored at -70°C until needed.

6.1 Treatment of plasma samples

The patient samples were thawed on the day of the cortisol and melatonin ELISA assays and diluted 100 fold with N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES buffer; 0.1 M; pH 7.5) supplemented with 0.1% (w/v) bovine serum albumin (BSA).

6.2 Hormone (Cortisol) plasma concentration determinations

6.2.1 Patient cortisol level quantification

The cortisol steroid hormone plasma levels were determined by enzyme-linked immunosorbent assay (ELISA) using a 96-well plate precoated with anti-cortisol IgG (ab10866 Cortisol ELISA Kit, Abcam) according to manufacturer instructions (Abcam, 2018). The kit was stored at -4°C and the samples at -70°C until required.

6.2.2 ELISA Assay

All reagents and samples were allowed to equilibrate to ambient temperature before being used. The 10x washing buffer was diluted with Milli-Q water to a 1x working solution and stored at 4°C until required.

The diluted samples and five cortisol standards (20 µl) were added to duplicate wells before the cortisol-horseradish peroxidase (HRP) conjugate (200 µl) was added to all the wells. Controls

included a manufacturer's cortisol control, background blank (with stop solution-0.16M sulfuric acid), as well as a substrate blank (no cortisol-HRP). The plate was covered with tin foil before being incubated for 1 hour at 37°C (Labotech Inco Therm Incubator). After incubation, the contents were aspirated before all wells were washed three times with the 1x washing buffer (300 µl) to remove unbound material. After each wash, the remaining fluid was removed by tapping the upturned plate onto tissue paper before the next wash.

After ensuring as much of the residual washing solution had been removed from all wells, 100 µl of 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate was then added. The plate was left to incubate at room temperature in the dark. The reaction was terminated by addition of a 100 µl stop solution that halted the colour development and produced a colour change from blue to yellow. The intensity of signal is inversely proportional to the concentration of cortisol in the patient sample. The absorbance at 450 nm was measured on a UV-Vis plate spectrophotometer (Labsystems iEMS Reader MF) with Ascent™ software version 2.6 within 5 minutes of adding the stop solution.

6.2.3 Data Analysis and Interpretation

A standard curve was constructed of absorbance (450 nm) versus log concentration of cortisol standards (cortisol concentrations: 0, 10, 50, 150, 500 ng/ml) using GraphPad Prism software version 5 (Figure 6.1). The concentration of patients' cortisol levels in plasma were extrapolated from the standard curve constructed and the 100x dilution factor accounted for.

The presence of any corticosteroid medication in the patients' samples would have competed with the added cortisol-HRP for antibody binding. Any possible medication such as cortisol, prednisolone, cortisone, prednisone, spironolactone, DHEA-S or dexamethasone, which could interfere with the assay, was recorded. The data for patients on haloperidol with or without tramadol showed varying results (Table 6.1 and 6.2).

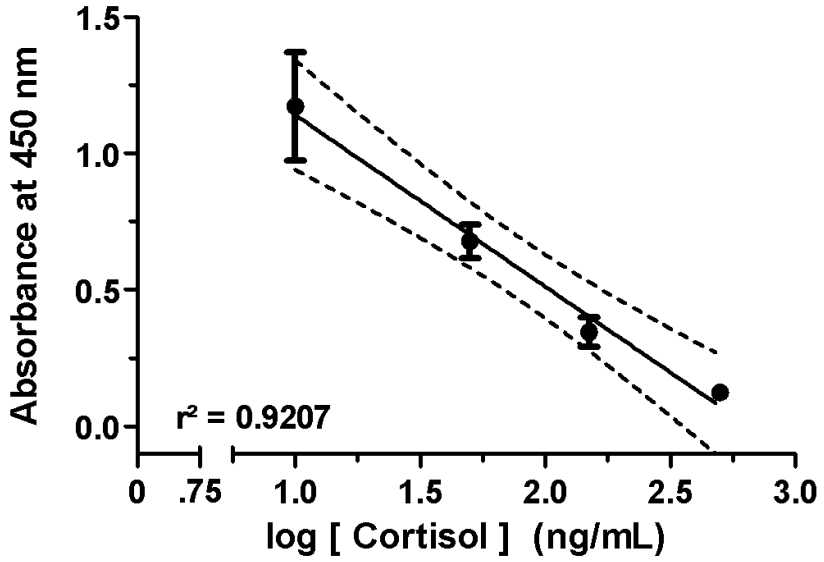


Figure 6.1: The standard curve of cortisol constructed from the standards supplied in the cortisol ELISA kit to determine the concentration in the patient samples from the linear regression line.

Table 6.1: Cortisol and melatonin levels in the haloperidol only group.

Sample Code	Cortisol Mean ±SD (ng/ml)	Melatonin Mean ±SD (ng/ml)
A029	26.4±14.1	3.3±0.9
A034	110.4±47.6	12.9±9.9
A062	12.8±5.2	4.6±1.4
A070	53.9±9.5	6.4±0.0
A076	6.7±0.5	2.8±0.2
Minimum	6.7	2.8
Maximum	110.4	12.9
Overall Mean	42.02	5.9
SD	42.3	4.1

Table 6.2: Mean cortisol and melatonin concentrations in the non-Haloperidol/ Tramadol group.

Sample Code	Mean Cortisol Levels (ng/ml)	Mean Melatonin Levels (ng/ml)
A051	38.4±19.6	21.1±1.2
A058	19.2±2.6	38.7±4.9
A065	205.1±86.9	356.6±0.0
A067	29.7±10.3	4.4±0.5
A068	11.0±5.0	15.2±0.9
A084	19.1±9.5	2.4±0.3
A087	865.1±369.8	75.9±7.3
A088	402.3±63.7	59.6±2.8
A089	58.9±7.53	53.9±2.1
A091	30.9±16.6.0	46.9±0.9
Minimum	**11.0**	**2.4**
Maximum	**865.1**	**356.6**
Overall Mean	**167.9**	**67.5**
SD	**274.43**	**104.47**

6.3 Patient melatonin level quantification

The melatonin concentration was determined using the melatonin ELISA (ab283259) kit, which consisted of a 96-well plate precoated with a rabbit melatonin monoclonal antiserum bound to goat anti-rabbit gamma globulin (GARGG) for which melatonin in the samples/standards competed with the melatonin-HRP (Abcam, 2018). The kit was stored at
-4°C and the samples at -70°C until required.

6.3.1 ELISA Assay

The patients' samples were prepared as in Section 6.1 above and were thawed on the day of the assay. All samples and reagents were allowed to equilibrate to ambient temperature before being used. The 10x washing buffer was diluted with Milli-Q water to a 1x working solution and stored at 4°C until required.

The undiluted patient samples, control 1 and 2, along with the six melatonin standards (25 µl) were added to duplicate wells before the melatonin-HRP conjugate (50 µl) was added to all the wells. The anti-melatonin solution (50 µl) was added to all well before the plate was covered with a plastic sealer and incubated for 2 hours at room temperature on an orbital shaker (Ohaus orbital shaker) at 25-500 rpm. After incubation, the contents were aspirated before all wells were washed three times with the 1x washing buffer (300 µl). After each wash, the remaining fluid was removed by tapping the upturned plate onto tissue paper before the next wash.

After ensuring as much of the residual washing solution had been removed from all wells, 125 µl of the TMB (TetraMethylBenzidine) substrate was added to each well to be catalysed by the HRP to produce a blue colour. The sealed plate was incubated for 30 minutes at ambient temperature in the dark, before the reaction was terminated by addition of 125 µl stop solution that halted the colour development and produced a colour change from blue to yellow. The intensity of signal is inversely proportional to the concentration of melatonin in the patient sample. The absorbance at 450 nm was measured on a UV-Vis plate spectrophotometer (Labsystems iEMS Reader MF) with Ascent™ software version 2.6 within 5 minutes of adding the stop solution.

6.3.2 Data Analysis and Interpretation

A standard curve was constructed of absorbance (450 nm) versus log concentration of melatonin standards (melatonin concentrations: 0, 3, 10, 30, 100, 300 pg/ml) with GraphPad Prism software version 5 and using the sigmoid dose response equation (Figure 6.2). The concentration of patients' melatonin levels in plasma were extrapolated from the standard curve constructed above with the final concentrations expressed as pg/ml.

The presence of medication that could have cross reacted with the antiserum was noted; for example, spironolactone or increased levels of serotonin or 5-methoxytryptamine (Abcam, 2021). Table 6.3 reports on the cortisol and melatonin level among patients receiving only tramadol

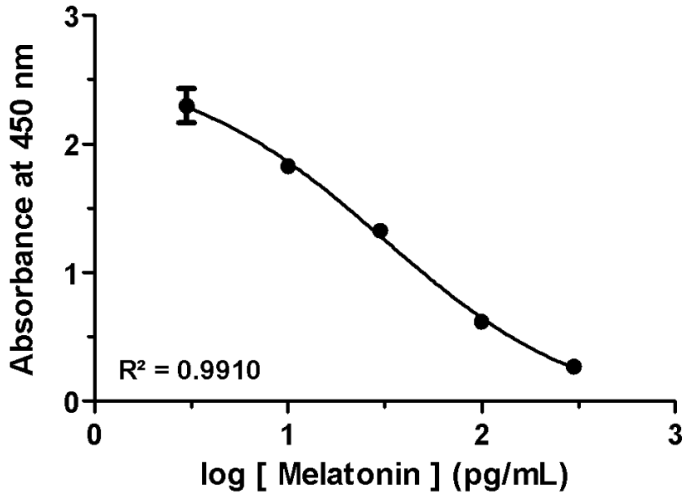


Figure 6.2: The standard curve of melatonin constructed from the standards supplied in the melatonin ELISA kit to determine the concentration in the patient samples from the sigmoid dose response equation.

Table 6.3: Tramadol only group

Sample Code	Mean Cortisol Levels (ng/ ml)	Mean Melatonin Levels (ng/ ml)
A015	86.5±14.2	66.8±12.3
A020	232.5±27.6	219.6±2.7
A026	28.9±10.2	3.9±0.6
A038	3.9±2.2	2.2±0.3
A045	43.1±9.1	2.6±0.0
A048	17.8±2.5	13.9±8.0
A049	141.2±3.5	14.5±4.1
A050	7.8±3.2	226.3±33.1
A052	2.9±0.6	17.4±0.4
A053	173.9±46.5	11.4±0.1
A054	44.9±1.9	15.6±0.7
A055	43.2±9.9	3.9±0.9
A056	14.9±3.4	43.90±1.2
A057	19.1±4.7	3.2±2.0
A063	10.8±2.5	2.5±0.6
A064	10.3±5.5	392.5±2.4
A083	326.7±1.7	23.4±6.3
A092	96.8±29.8	9.1±7.7
A094	25.5±0.3	7.0±3.6
A096	15.9±8.0	15.2±2.8
Minimum	2.9	2.2
Maximum	326.7	392.5
Overall Mean	67.3	54.8
SD	87.6	103.1

6.4 Discussion of the main findings of cortisol and melatonin using spectrophotometry and ELISA

Hormone measurement in routine clinical laboratories relies largely on immunoassays, owing to advantages such as low cost and technical ease.

A total of 94 agitated critically ill patients with self-reported sleep occurring in the ICU, with an average sleep length of 3.5 days (1-11 days) with sleep complaints or sleep disorders were enrolled. Cortisol and melatonin are clinically important for diagnosing sleep and mood disorders.

Cortisol, a steroid hormone secreted by the adrenal gland has been associated with physical and emotional stress as well as the circadian rhythm (Wuff *et al*., 2012). While melatonin is a hormone primarily secreted by the pineal gland, it is clinically important for the diagnosis of sleep and mood disorders and affects the circadian rhythm and many other physiological functions (Keijzer *et al* 2014).

A validated sampling method for plasma is important since incorrect sampling may significantly influence the results. Because of the circadian variation of cortisol and melatonin secretion, the deviation from a fixed daily sampling procedure may increase variation of the results. In some cases the clinical interpretation may not be possible without exact knowledge of the collection time of the sample. A plasma melatonin and cortisol sample represents the production of cortisol and melatonin over a fixed time period which may therefore better reflect cortisol and melatonin production than a single serum sample (Alhajj *et al*., 2023). Repeated 24-h plasma collections may, however, be difficult in clinical practice. Accurate collection of plasma needs good cooperation with the patient. Therefore, in many studies, the mean of two to three 24-h plasma collections are used for reliable estimation of the average daily cortisol and melatonin (Turpeinen *et al*., 2013). However, for the sake of this study only 24 hour sample was used.

Comparing the plasma **am** cortisol level with that of **pm** melatonin level among critically ill ICU patients, shows that sixteen patients (n= 16) were in cortisol group while nineteen patients (n= 23) were in melatonin group respectively. The results of the diurnal variation of cortisol and melatonin provide biochemical correlates for our clinical data.

A total of six patients (n= 6) were on treatment with dexamethasone, hydrocortisone or methylprednisolone. Only two of these patients demonstrated plasma levels below the manufacturer's reference range of 0-50 mcg/ dl. A study by Lee *et al*. (2004) reported that drugs

with similar structural architectures with steroid receptors such as cortisol can cause significant interference with the immunoassay. Other anabolic steroids and synthetic glucocorticoids were also reported to affect the assay (Lee *et al*., 2004).

The minimum and maximum mean cortisol (**am**) levels among patients were 0.595±0.068 mcg/ dl and **402.272±63.696** mcg/ dl; while the minimum and maximum mean melatonin (**pm**) levels recorded were 1.406±0.547 pg/ ml and **647.194±11.676** pg/ ml respectively. Only three patients (n= 3) showed remarkably high **am** cortisol levels (mean, **499.944±153.679** mcg/ dl) above the reference range provided by the manufacturers of 60-230 mcg/dl; while three other (n= 3) patients showed very high levels of **pm** melatonin with an average of **393.355±20.484** pg/ ml compared to 20 patients with lower **pm** plasma melatonin levels (mean, 17.176±2.879 pg/ ml). An increase in melatonin levels during sleep deprivation is associated with increased adrenergic stimulation as reported by Klein and Walter (1973).

In normal adults, melatonin secretion commences at night at around 21:00 maximum secretion as observed at about 22:00 and 4:00 respectively (Riutta *et al*., 2009). Inhibition of melatonin endogenous hormone begins around 19:00 and 21:00 coinciding with the peak level of endogenous cortisol (Riutta *et al*., 2009). However, in this study; transition from dim light to bright light in the morning may be inhibited because of constant lighting in the ICU, in addition to other factors that affect sleep in the ICU (section 4.11). All the patients with high hormone levels (Table 6.1) (cortisol, 232.5±27.6 and 326.6±21.7; melatonin, 226.3±33.1 and 392.5±2.4) were managed as a case of acute kidney injury and have below average sleep quality of <50 mm (section 4.12). However, the acute kidney injury was secondary to acute poisoning which was also reported by some studies to increase the levels of cortisol and melatonin respectively (Guven *et al*., 2022).

Circadian imbalances from dysregulated cortisol and melatonin can lead to day time sleepiness and brain processing issues such as decreased alertness and problems with memory.

Critically ill ICU patients are at risk of circadian disruption and the commonest manifestation is delirium (McKenna *et al*., 2018). Many studies have been designed to investigate the relationship between circadian rhythm and delirium in ICU patients (McKenna *et al*., 2018). However such studies have a lot of limitations. A study to determine melatonin levels over three consecutive days in eight ICU patients found that alteration of the melatonin rhythm may impair sleep and ultimately lead to delirium (Olofsson *et al*., 2004). However, a limited number of participants have been reported to affect the rationality of the study conclusion. Another study tested urine aMT6s, the

active metabolite of melatonin, which was used as a substitute for melatonin in a simulated ICU ward, and reported a significance difference in nocturnal urine melatonin of aMT6S (Hu *et al*., 2010). Furthermore, the study was later discredited because the urinary melatonin secretion is not a reliable measure of melatonin in critically ill ICU patients.

In the current study, venous/ arterial blood was used to test the circadian rhythm of cortisol and melatonin secretion in critically ill ICU patients. Melatonin plays a very important role in the regulation of the sleep–wake cycle (Oxlund *et al*., 2021). In this study most of the ICU patients showed low **pm** levels of melatonin. Several studies reported controversial results between delirium patients and non-delirium patients (Li *et al*., 2023). For example, a study reported by Oxlund *et al*. (2021) measured melatonin at 14:00, 22:00 and 3:00 daily for three consecutive days, and reported no significant effect of suppressed melatonin concentration between delirium patients and non-delirium patients (Li *et al*., 2023). Another study on saliva melatonin collected from patients on day 1 of admission (7:00, 21:00) until discharge, found no melatonin rhythm in delirium patients and mean melatonin levels were decreased among in-patients. The study also reported lower night salivary melatonin level in ICU patients (Angeles-Castellanos *et al*., 2016).

Another study on the morning urine samples on the day of surgery and three days after surgery used the 6-sulfatoxymelatonin (6-SMT)/ creatinine ratio to estimate urine 6-SMT levels. The group with high 6-SMT showed a threefold ratio increase postoperatively with cognitive dysfunction among these patients (Wu *et al*., 2014), hence they demonstrated an association between delirium and circadian rhythm. Compared with the above mentioned study however, this study involved differences in sample collection timing, i.e. at 8:00 and 4:00 and this was observed to influence the results.

In the current study the **am** cortisol and **pm** melatonin levels of patients that were on haloperidol only and those that are not on haloperidol as well as tramadol were also compared. . The overall mean cortisol levels for patients on haloperidol were 42.06±42.31 ng/ml compared to the overall mean value for patients that were neither on haloperidol nor tramadol group (167.96±274.43). This indicated that patients in the latter group showed significantly higher cortisol levels compared to the former. A systematic review article reported that most of the studies in patients with stress suggested that atypical antipsychotics such as risperidone reduces cortisol levels to a greater extent than typical antipsychotics like haloperidol (Borges *et al*., 2013). Another study reported that a single oral dose treatment with haloperidol resulted in prolonged reduction in salivary cortisol

levels (Handley *et al*., 2016).

The main mechanism of action of haloperidol is blockade of mesolimbic dopamine receptors by acting on both D_1 and D_2 receptors, as it exerts a stimulatory role on the activation of HPA (Sullivan *et al*., 2006; Belda *et al*., 2009). However, high antagonism at D_2 receptors in the striatum following treatment with antipsychotics could directly reduce ACTH and cortisol levels (Oswald *et al*., 2005; Wand *et al*., 2007). Another underlying mechanism that haloperidol is to reduce cortisol levels through stimulation of pro-inflammatory cytokines, thereby reducing the plasma levels of ACTH and cortisol respectively (Sugino *et al*., 2009; Handley *et al*., 2016).

Comparing the cortisol and melatonin levels of patients on the haloperidol only group shows that there is reduction in melatonin levels (Table 6.3) (Overall mean, 5.99±4.09 ng/ ml) with RCSQ score of 26.80 mm. This below the average score as defined as 26.80 mm and hence described as poor sleep among patients on haloperidol (section 4.12). The result also indicates rhythmicity or sleep wake disorder in the melatonin levels among the non-haloperidol group of patients.

Patients on treatment with only tramadol showed concentration levels within the reference range limit of 67.32±7.9 pg/ ml (range, 2.0±7 – 326.0±65), also patients not on treatment with haloperidol/ or tramadol. However, the RCSQ score for patients on tramadol was above average (RCSQ, 54.0 mm), indicating moderate sleep. The overall mean melatonin levels among the tramadol group were 54.78±103.11 ng/ ml compared to the non-tramadol/ or haloperidol group (overall mean, 67.48±104.47 ng/ ml).

It is estimated that 50-88% of patients suffering from chronic pain experience inadequate sleep which can be observed among ICU patients (Segal *et al*., 2018). However, patients on low dosages of tramadol report a subjective increase in sleep (El wasify *et al*., 2018). Recent studies suggested that both pain and sleep are regulated by the circadian clock and genes (Wei *et al*., 2018). Most of the studies of pain regulation by circadian clock are being done in animal models; however, there is an urgent need to have an understanding of circadian pain regulation in humans (Smith *et al*., 2000). A study to investigate the effect of melatonin administration on perioperative cortisol levels and opioid consumption in total knee arthroplasty (TKA) concluded that melatonin does not affect postoperative opioid consumption or cortisol levels following TSA (Kohler *et al*., 2020). A study of sleep characteristics in patients with tramadol dependence revealed that patients with tramadol addiction have a disrupted sleep pattern and quantity of sleep, combined leading to less sleep efficacy and increase arousal from sleep as well as lower SWS and REM sleep (Abdallah *et al*., 2020).

These problems were linked to the usage and increase in tramadol dosage (Abdallah *et al*., 2020). A randomised cross-over trial in healthy volunteers reported that a single dose of tramadol (50 mg) disturbs sleep in the night of drug administration, while in volunteers, a single dose of 100 mg leads to sleep disturbance in both the night of drug administration and in the subsequent night (Walder *et al*., 2001). Contrary to the current study where most of the ICU patients were on 50 mg of tramadol, depending on the severity of the pain, which may explain the reason why the specific study cohort had moderate sleep (Table 4.9).

CHAPTER SEVEN

SUMMARY AND DISCUSSION OF THE MAIN FINDINGS

7.1 INTRODUCTION

In the previous chapters (chapter 1, 2, and 3), both descriptive and comparative analyses were employed to describe and synthesize the data, and the interpretations of the research findings were presented. The main purpose of these respective chapters was to describe and explore the clinical profile of patients admitted to the Intensive Care Units at a university-affiliated, public sector and tertiary level hospital in the Gauteng province in South Africa.

Chapter six and seven of this study present a summary, discussion and conclusion of the findings. This is followed by limitations of the study, recommendations based on the findings.

7.2 SUMMARY OF THE STUDY

7.2.1 Purpose of the study

The purpose of this study was to determine the effect of haloperidol on stress hormones in ICU patients with delirium and perceived quality of sleep whilst in an ICU at an academic level 1 public sector hospital in Johannesburg, Gauteng province, South Africa.

7.2.2 Objectives of the study

The research objectives for this study were divided into two sections, being the clinical (chapter four) and laboratory (chapter five and six). The laboratory aspect was carried out in the laboratory; blood plasma and urine samples of the patients were used so as to quantify the levels of haloperidol using HPLC analysis and relate these levels to the stress hormone levels in the plasma via ELISA. On the other hand, the clinical aspect of the study involved three different questionnaires that were administered to the patients while discharged from the ICU to the ward or at their first follow-up visit. Also to investigate and determine the prevalence and severity of anxiety, depression and posttraumatic stress symptoms they experienced.

7.2.3 Methodology

A sample size was of one-hundred (n=94) to ensure a power of 95% accuracy. A p value of 0.05 was declared statistically significant testing was considered appropriate. However, six patients were excluded as they did not want to consent and hence 94 patients were enrolled in the study.

Haloperidol plasma levels were determined using HPLC analysis, while ELISA kits were used to determine melatonin and cortisol levels using patients' blood samples. To assess delirium among ICU patients a validated and well published CAM-ICU screening checklist was utilised (Appendix A).

A question and answer session (5-10 minutes) was conducted between patient and researcher using the DASS instrument as well as the RCSQ questionnaires in order to obtain patients' response and perception. In order to determine severity of illness, the SAPS II psychological score was utilised (Appendix A).

Prior to commencement of the study, ethical clearance was obtained from the Human Research Ethics Committee (Medical), (**M180747**, *Appendix* E). Permission was also obtained from the Faculty of Health Science Postgraduate Committee (*Appendix* C). Permission was also obtained from the chief executive officer of the Charlotte Maxeke Academic Hospital, the Director of the ICU, doctors and nursing managers.

A pilot study was carried out prior to the commencement of the main study in order to test the outcome measures, wording, information letter and informed consent. A prospective study with a quantitative approach with data collected at multiple points during the patient's admission was used to meet the objectives of this study.

Data collection took place over the period November 2019 December 2019 to March 2020. Descriptive and inferential statistics were used to analyze the data.

7.3 DISCUSSION OF THE MAIN FINDINGS

7.3.1 Related to the age distribution

In this study, the mean age was 46.19 years (SD 17.19); the ranges for the age were from 19 to 85 years. Findings indicated a younger age than similar studies in other parts of the world (Nouira *et al*., 1998). The study by Nouira and Arabi *et al*., in the *Northern African* region reported a mean age

with compared to our study of 45.8 to 48.3 years and 49.1 years, respectively (Nouira *et al*., 1998, Arabi *et al*., 2001). However, a study conducted in the western part of the world (United State of America) reported a much higher mean age of 59.1 and 61.7 years, respectively compared to our study (Iapichino *et al*., 2004, Zimmerman *et al*., 2006). The higher age might be due to the higher number of elderly people in that part of the world (USA) compared to the African population where the majority of the population were younger (www.un.org/esa/population/publications/WPA2009. Accessed 26 April 2020).

7.3.2 Related to gender distribution

Males accounted for 51.06% (n=48) and females 48.9% (n=46) of the total population (n=94), and the ratio of male to female patients was 1.04:1. However, inter-diagnosis analysis indicated a higher male to female ratio among medical emergencies, suicidal attempts and others which were 1.67:1, 1.08:1 and 1.06:1, respectively. Similarly, a lower (0.5:1) female to male ratio was indicated in patients with carcinomas and acute abdomen which is consistent with some ICU studies (Livingston *et al*., 2000). Many studies reported males as the most dominant gender in the ICU, which is consistent with the current finding in our study (Livingston *et al*., 2000; Arabi *et al*., 2001). However, a study conducted in Tunisia showed up to 55.9% of their sample populations were males and 44.1% females, which is similar to the finding in this study (Nouira *et al*., 1998).

7.3.3 Related to reason of admission

A higher percentage of patients (37.60% (n= 40)) was diagnosed with medical emergencies and 21.62% (n= 23) were suicidal attempt cases. Of all the patients, acute abdomen was slightly higher (8.46%; n= 9) than carcinomas, which was 7.52% (n= 8). Other cases constitute 13.16% (n=14). In this study, the reason for admission distribution for medical emergencies is significantly higher than other emergencies which is in line with many foreign studies where they found higher surgical than medical emergency cases (81.3% versus 18.7%) (Capuzzo *et al*., 2000). Similarly, a South African study conducted in general ICU found the number of surgical and medical emergencies to be similar (Schmollgruber *et al*., 2015), conversely, in an Italian study reported a contrast result to the aforementioned studies, they found a much higher number of medical emergency cases than surgical emergencies (69.4 vs. 30.6%) (Zimmerman *et al*., 2006). In this study,

the reason for admission for surgery-related emergencies was slightly higher than other emergencies; similar studies conducted in the western world reported 65% of surgery-related emergency cases in a multicenter study (Rosenberg *et al*., 2000). Nouira *et al*. (1998) also reported a much higher percentage of patients (63%) compared to the current study. The medically-related emergencies in this study was higher than many previously reported studies (Capuzzo *et al*., 2000; Nouira *et al*., 1998) where they reported 1.7% and 11%, respectively. However, Rosenberg *et al*. (2000) and Livingston *et al*. (2000) reported similar categories compared to this study, respectively (23.7% vs. 21.3%).

7.3.4 Related to severity of illness

The mean SAPS II score was 40.93 (SD 14.23) points for patients' admission to ICU, and range was 12-82 points for the total sample population (n=94). Of the total sample population (n=94), medical emergency patients had a higher (45.11; SD 13.1) total SAPS II score followed by lower scores of 41.2 (SD 11.42), 40.39 (SD 12.24) and 35.63 (SD 12.52) indicated in other surgery related being, acute abdomen and carcinoma care patients, respectively. Moreover, the observed hospital mortality was lower than that predicted by SAPS II (14.9%, n= 14; vs. 30.8%) for the total population (n= 94). This indicated that predictions made by the SAPS II scores for the total population (n= 94) to be twice the actual mortality, probably due to the higher number of medical emergency cases which has been shown to carry a higher risk of mortality (Lee *et al*., 2015). Findings in this study related to SAPS II scores are consistent with the patterns of some studies conducted in other foreign countries (Capuzzo *et al*., 2000). The total mean SAPS II score found in this study (30.8%) was slightly higher than some studies abroad, where they found a total mean SAPS II score of 25.7 and 33.1 respectively (Katsaragakis *et al*., 2000), whilst one study conducted in China showed a higher SAPS II mean score compared to this study (41.5; SD 21.3) (Kwok *et al*., 2005). In this study, the total mean SAPS II score for surgery related emergency cases (41.2; SD 11.42) was slightly higher than total mean SAPS II score reported by some studies (39.0 vs. 34.2), respectively (Apolone *et al*., 1996, Nogueira *et al*., 2009).

7.3.5 Related to length of stay

The mean length of stay for the total population (n=94) was 3.4 days and a range of 9 days. Furthermore, the majority 92% (n=97.9) frequency responses indicated length of stay was in the 1-

7 days category. By contrast, only 5.3% (n=5) were in the category of > 11 days, which was categorized as a long-term ICU stay (Adamson, 2004). However, an observational study by Granholm et al., showed that patients with longer ICU length of stay, indicated predictive performance of admission-based severity scores that may deteriorate, compared to patients with shorter ICU length of stay, hence the majority of patients in this study had a shorter length of stay of 3.4 days (Granholm *et al*., 2019). Findings in this study are consistent with some studies, where they reported a mean length of stay of 3.82 days (Zimmerman *et al*., 2006). However, many studies reported a much higher ICU length of stay of 4.4, 5.8, 10.8 and 16.55 days which are higher than what is reported in this study (Iapichino *et al*., 2000; Capuzzo *et al*., 2004; Apolone *et al*., 2000, Nogueira *et al*., 2009). The findings for length of stay for this study are longer than some studies where they reported a mean length of stay of 2.9 days (Seferian *et al*., 2006). Similarly, a study conducted in Germany among surgical intensive care unit patients reported a median length of stay of 1-3 days for their total sample population (Sakr *et al*., 2010).

7.3.6 Related to patient outcomes

With regard to the differences between ICU survivor and non-survivor population groups, there is no statistically significant difference in SAPS II score between ICU non-survivors and ICU survivors (p=0.059; CI 0.34-4.33). Of the total SAPS II score items, only two items were statistically significantly different (p<0.05). Included were age and ventilation PaO_2/ FiO_2 ratio, respectively. Of the 15 SAPS II items, only three scores were statistically significantly (p<0.05) different at the sub-item level scores. Included were also age and ventilation PaO_2/FiO_2 ratio. These sub-level items showed a pattern of the higher the age of the patients, the higher the likelihood of having poor patient outcome and the poorer the ventilation with oxygen, the poorer the patient's outcome respectively. Findings in this study are consistent with the distribution trends in a study conducted in Germany where they investigated the performance of SAPS II score among a large cohort of surgical intensive care units. They reported a poor performance in general estimate but excellent performance at sub-group level (Sakr *et al*., 2010). However, when the SAPS II score was compared with the TISS-28 and the APACHE III scoring tool, many studies reported a good correlation between the two (Kwok *et al*., 2005; Metnitz *et al*., 1999). However, some reported that the SAPS II score was by far superior over the APACHE III score when estimating mortality among ICU patients (Livingston *et al*., 2000).

7.3.7 LABORATORY RESULTS AND PATIENT OUTCOME

7.3.7.1 Patients' response: Biochemical and hematological results

Leukocytosis was recorded in 35.1% of our study population and the count was higher on the 1st date of admission compared to date 2, respectively (65.5 x 10^9 /L vs. 30 x 10^9 /L) (range, 4.0-11.0 x 10^9 /L). Sepsis is common in ICU and usually complicated with organ dysfunction and difficult treatment (Anderson BJ *et al*., 216). Sepsis is the profound dysregulated inflammatory state driven by the presence of infection. The process begins with focus infection, stimulating the body to respond by releasing toxic products; pro- inflammatory mediators are released ultimately causing tissue injury and organ dysfunction (Bone *et al*., 1996, Pinsky *et al*., 1989). However, advanced age (>65 years), diabetes mellitus, immunosuppression and recent hospitalization has been shown to be associated with severe illness and increased mortality in patients with sepsis (Martin *et al*., 2916, Sands *et al*., 1997). Findings in this study show similar trends with a study conducted among critically ill patients with acute kidney injury, where they found sepsis among 47% of their sample population (Markovich *et al*., 2020). A pilot observational study also reported similar statistics among lung and abdominal septic patients, compared to a higher percentage (>80%) among genito-urinary, skin and soft tissues infected patients, respectively (Jeganathan *et al*., 2017).

In comparison between ICU non-survivors and ICU survivors, the white blood cell count was found to be elevated among patients that died compared to those that survived, with no statistically significant difference between the two (p=0.087) (Table 4.16). This is consistent with a USA study that found higher mortality among patients with multiple organ failure due to sepsis compared to those with no single organ involvement (Jeganathan *et al*., 2017).

In this study, 21.3% of the patients had severe renal impairment (acute kidney injury; 15.9%, chronic kidney disease 5.3%) with statistically significant association between ICU non- survivors and ICU survivors, respectively ($p<0.05$) (Table 4.16). A study conducted in Serbia reported similar findings, where they reported that up to 20% of the study population were having AKI with less than half of the patients accounting for CKD, however, pre-renal cause was reported to account for more than 80% of the etiology (Markovic *et al*., 2020). This study also found age and comorbidities to be associated with poor outcome and higher mortality among these patients. Only few of the AKI patients tend to improve with conservative treatment (9.52%) while the majority of the patients will require renal replacement therapy or dialysis as reported by some studies (90.48%) (Markovic *et al*., 2020).

Platelet count was found to be within the normal range between 1st day and date 2 of admission, respectively (200.5 vs. 253.0 x 10^9/L) (range, 137-373 x 10^9/L). However, platelet count showed a statistically significant difference between ICU no-survivors and ICU survivors ($p<0.05$). Thrombocytopenia is common in critically ill patients and has been associated with poor clinical outcomes with potential to be used as a prognostic therapeutic target for patients with critical illness (Marvi *et al*., 2020). A USA study showed a similar trend of findings with this study, where they found a median ICU admission platelet count of 214 x 10^9/L (range, 157 x 10^9/L - 276 x 10^9/L) (Marvi *et al*., 2020). A study conducted in Sudan also showed a similar ICU admission platelet count compared with this study of 237 x 10^9/L, however, the study highlighted the importance of prompt response when abnormal platelet count was detected among ICU patients (Rahama *et al*., 2019). The liver function test results in this study indicated varying results. Of the 94 sample population 24.5% demonstrated an abnormal LFT at the point of admission. The majority of abnormalities were less than twice the upper limit of normal for 1st day of admission and date 2, respectively (ALT, AST, albumin and total bilirubin) (AST, 55 vs. 35 µ/L; ALT, 35 vs. 35 µg/L) (range, 5-40 µg/L). Similarly, the same finding was obtained with albumin and total bilirubin, respectively (albumin, 28 mg/dL vs. 23 g/dL) (range, 35-52 mg/dL) (total bilirubin, 20 vs.18 mg/dL) (range, 0-21 mg/dL) (Table 4.15). Findings in this study are in line with findings by a prospective, observational study conducted by Thomson *et al*. (2009) that investigated liver function tests on intensive care units. Among the four parameters, only albumin demonstrated a weak statistically significant difference between the ICU non- survivors and survivors ($p<0.05$) (Table 4.16).

7.3.8 DRUG TREATMENT OPTIONS IN ICU

Intensive care units (ICUs) care for the most critically ill patients. Therefore, a number of drugs are used to treat these patients. Some of the drugs are therapeutic, and others are prophylactic in nature.

7.3.8.1 Antipsychotics and Benzodiazepines

In this study typical antipsychotic haloperidol was predominantly used for the treatment of delirium (n=20, 21.3%) compared to, two patients treated with atypical antipsychotic risperidone (n=2, 2.1%). Both typical and atypical antipsychotics are used in clinical practice to manage delirium. The typical antipsychotic most often used is haloperidol whilst the atypical ones include risperidone,

olanzapine, clozapine and aripiprazole (Svoboda *et al*., 2023). The American Psychiatric Association Guidelines (Trzepacz *et al*., 1999) suggest using haloperidol, it is the most studied for treatment of delirium (Hatta *et al*., 2014) and has many years of clinical experience. Recently however, prospective studies has provided evidence of superiority of atypical antipsychotics when it comes to rapid onset of action and lower incidence of extrapyramidal symptoms (Kishi *et al*., 2016, Wilson *et al*., 202012). A survey of behaviour and attitudes of 1, 384 healthcare professionals found over 86% of the delirium patients to be treated with haloperidol and nearly 40% reported using atypical antipsychotics, however, there are no large, placebo-controlled trials examining the efficacy of these medications in treating ICU delirium (Patel *et al*., 2009).

Patients in this study were treated with antipsychotics based on the presentation of delirium symptoms and in accordance with guidelines by the National Institute for Health and Care Excellence (South Africa) that being that patients suffering from serious distress from these symptoms or when patients poses a threat to themselves or others and finally when patients impeded on the essential aspects of their medical care. Patients with delirium symptoms due to substance withdrawal were not administered antipsychotics as outlined in the criteria of the study. However, treatment with antipsychotics in the management of delirium remains controversial (Karunarathna *et al*., 2023). While many studies suggest these drugs are beneficial for the patients, contrary findings have also been reported (Bourne *et al*., 2008, Campbell *et al*., 2009, Devlin *et al*., 2011).

In this study, the antipsychotic was stopped as soon as the delirium symptoms resolved, in most cases in 3 to 5 days except among organophosphate poisoning patients in whom it can be extended for a longer period. Most antipsychotic medications are known to prolong QTC- interval and this poses a risk of torsades de pointes (Karunarathna *et al*., 2023). However, in this study, despite this risk, its use in delirium patients proves to be effective and safe as reported by other studies (Bourne *et al*., 2008, Campbell *et al*., 2009, Devlin *et al*., 2011). In this study, antipsychotics were more likely to be used among medically-related emergencies (n= 40, 37.6%) compared to suicide attempted patients (n= 23, 21.62%) followed by other emergencies (n= 14, 13.16%), acute abdomen and carcinoma patients to a lesser extent (n= 9, 8.46%, vs. n= 8, 7.52%).

The Richmond agitation and sedation scale (RASS) was used for assessment of sedation which was reported to have high reliability and validity (Ely *et al*., 2003). Benzodiazepines were the most commonly used sedative medications (6.4%), and <1% propofol and ketamine, respectively.

Benzodiazepines were extensively studied and reported as a risk factor for developing delirium in the ICU (Pandharipande *et al*., 2006). Recently, some studies have reported a reduction in the use of benzodiazepines as primary mode of sedation in ICU patients (Pandharipande *et al*., 2007). The results of this study highlight the differences between the perception and detection of delirium as an important outcome of critical illness and current practice in delirium screening, monitoring and treatment. Physicians and intensive care nurses recognize delirium as a common occurrence in the ICU, but their perceived problem prevalence of delirium continues to be lower than the results coming out of the conducted studies in the ICU environment (Kang *et al.,* 2023). With advancement of critical care medicine the number of people using a validated screening tool for delirium (mainly CAM-ICU and ICDSC) has increased significantly (van den Boogaard *et al*., 2024). In the current study this number is lower than expected. Hence more sensitization is needed to improve specific practices in delirium and sedation management.

7.3.8.2 Opiate analgesics

In critically ill patients opioids are most often used for acute pain treatment (Varga *et al*., 2023). A variety of opioids are used in clinical practice this being, morphine, codeine, pethidine, synthetic morphine analogues (fentanyl) and atypical opioids (tramadol) (Varga *et al*., 2023). The most common opiate analgesics used in this study are tramadol (63.8%) and morphine (27.7%), respectively. Tramadol is a centrally-acting analgesic with weak opioid effects used for moderate to severe pain (Grond *et al*., 2004). Adequate analgesia in critically ill patients ensures comfort and calmness which in turn allow health care providers to carry out procedures on patients with ease; however, it also promotes reduction in stress response related to severity of disease caused by trauma or inflammation (Grond *et al*., 2004).

Analgesia is an act of relieving or killing pain achieved through administration of analgesic drugs which act either peripherally or centrally (Sampson *et al*., 2020). It has been reported that about 45-82% of the critically ill patients in the ICU suffer from pain (Walder *et al*., 2002). Critically ill ICU patients are exposed to numerous activities that cause pain, as example, suctioning, endotracheal tube insertion and chest tube removal.

Pain has been linked to pathogenesis of delirium and anxiety in the ICU (Sampson *et al*., 2020). Appropriate analgesia appears to be obvious for these patients, although there is no scientific evidence to link the successful control of agitation or delirium with improved pain outcome (Hassan

et al., 1998).

7.3.8.3 Antimicrobial Agents

Antibiotic therapy is the cornerstone in the treatment of infection (Nyhoegen *et al*., 2023). The influence of adequate antimicrobial therapy on the prognosis of severe sepsis and septic shock has not been clearly proven (Rello *et al*., 1994; Lundberg *et al*., 1998). Early initiation of adequate antibiotic treatment in bacteremia patients was associated with a reduction in mortality rate (Cisneros *et al*., 1996; Pederson *et al*., 1997), however, this benefit was not observed in critically ill patients (Rello *et al*., 1994). In these patients, the fatality is probably related to the age of the patient and the presence of underlying disease and comorbidities (Angus *et al*., 2001; Pittet *et al*., 1993). In this study about 31.9% of the patients received antibiotics for treatment of one or more infection, the majority of them were surgery related emergencies, medical related emergencies and followed by acute abdomen and carcinoma patients, all received antibiotics. Co-amoxiclav (Augmentin®), ceftriaxone (Rocephin®), azithromycin and metronidazole were the most common antibiotics used in this study. The high incidence of ceftriaxone usage reflects the preference by the doctors for its use as a postoperative antibiotic. The prescription was solely done by rotating registrars and unit consultants. Antibiotics were administered intravenously by the nurses in the unit.

Empirical antibiotic treatment was not always the rule in the general ICU, however, the antibiotic stewardship protocol of the hospital was strictly adhered to. Only patients with positive culture were placed on antimicrobial agents. Studies showed that inappropriate empirical antimicrobial therapy was associated with a significant increase in risk of death and early initiation of adequate antibiotic treatment in bacteremia patients was associated with a reduction in mortality rate (Pittet *et al*., 1993). For patients suspected to have a focus of infection; culture was obtained from the focus site and samples were taken to the laboratory for identification. As example, culture of pus obtained from operating table, cerebrospinal fluid (CSF), urine or sputum from pneumonia or pulmonary tuberculosis patients. As presented by Richard *et al.* 2018*,* blood infection (due to devices), urinary tract infection (due to urethral catheter) and pneumonia (due to ventilation devices) account for more than two-third of the nosocomial infections in the ICU. The culprit organism reported includes *Staphylococcus aureus*, *Escherichia coli* and as the commonest causative organism respectively (Nyhoegen *et al*., 2023). However, there were no specific microorganisms associated with a higher death rate either in bacteremia or the site of infection.

7.3.8.4 Other drugs

Other drugs used in the ICU were either for the prophylaxis or therapeutic purposes. For example enoxaparin sodium (Clexane®,Antithrombotic agent) was administered to over 90.4% of the ICU patients for the prevention of deep venous thrombosis (DVT) because of long time inactivity and being bedridden predisposes patients to the risk of DVT.

Pantoprazole was used for the treatment of peptic ulcer symptoms common among post- operative patients and patients weaned from ventilators.

7.3.9 Anxiety, depression and PTSD

All the patients included in this study were interviewed following their subsequent discharge to the respective wards. Symptoms of anxiety were found in 45% of the study population, depressive symptoms were found in 25% and PTSD symptoms in 38% of the sample population at their first interview in the ward post-discharge from ICU (Figure 4.7). A significant number of patients who survive a critical illness show evidence of anxiety and depression up to 9 months after discharge and most of them also demonstrate some symptoms of PTSD. This has also been true with the South African patients irrespective of their ethnicity, age, gender and ICU type. A Delayed identification of individuals and subsequent follow up or psychological support, leads to patients that may experience chronic suffering with a serious impact on their quality of life, family members as well as their integration back into society and gaining employment.

7.3.9.1 Prevalence of anxiety and depressive symptoms

- **Anxiety**

The prevalence of anxiety symptoms in this study was high with close to half (45%) of the patients showing symptoms. Majority of the patients show clinical cases of anxiety as demonstrated on the HADS scale. These findings were also reported in other studies that use the HADS scale to report psychological sequencing of critically ill ICU patients (Bjelland *et al*., 2002). As opposed to other study findings, males have shown higher prevalence of anxiety in this study than women and slightly lower depressive symptoms. A previous study has repeatedly shown that psychological distress is more prevalent among women than men (Bryant *et al*., 2008). However, some studies

have shown no statistically significant differences between women and men regarding prevalence of depression as reported in this study (Stordal *et al*., 2001; Helvik *et al*., 2011). Higher prevalence of anxiety in this study is of concern (45%) as the significance of anxiety and its consequences is often ignored by medical professionals, as the anxiety did not show immediate consequences among many people after hospital discharge (Fawcett J., 1997).

- **Depression**

The prevalence of depressive symptoms was less than that of anxiety but still high, with slightly higher than one third of the study population (25%) showing symptoms according to the HADS depression scale classified as a clinical case of depression (Table 4.17 and Figure 4.7). Scragg *et al* (2001) reported a 30% prevalence of depressive symptoms above the score of eight on HADS. The findings of this study and the prevalence of depressive symptoms are thus similar.

- **Prevalence of PTSD symptoms**

The prevalence of PTSD symptoms directly related to the ICU experience, as measured by the ETIC-7 scale, is 38%. This is slightly above a third of the total sample population. These findings are similar to those reported by other studies (Mealer *et al*., 2007) that reported prevalence of PTSD symptoms (24%). Of the 38% with PTSD symptoms, eight of the patients had scores of 15 on the ETIC-7 scale, indicating severe symptoms. Three patients had a full score of 21. Twenty percent (20%) of patients were males and eighteen percent (18%) were females, respectively.

The prevalence of PTSD symptoms was high in this study and of major concern as the symptoms persisted over time. The concern is corroborated in the work of Mealer *et al*., 2014 that show that PTS symptoms may increase morbidity and mortality as well as increase the burden of care (Mealer *et al*., 2014). Hence it is important to screen and identify those at risk. Patients in ICU treated for HIV infections have also shown to account for the highest prevalence of PTSD (Martin *et al*., 2011). The black population had the highest prevalence of PTSD symptoms (32%) this is followed by white population with 5%. The Indian population had the lowest prevalence of 1% with only one patient in this category related to the study. With reference to studies, PTS symptoms exceeding three month duration after ICU admission tend to persist for a long time when not treated. Studies by Mealer *et al*., 2011 also observed that if treatment was not administered after 7 years, that this may progress to chronic suffering. Patients with PTSD symptoms scores of 8-15 on the ETIC-7 scale were likely to develop severe symptoms over time (Mealer *et al*., 2011). Some of the immediate

consequences of chronic PTSD symptoms include loss of day-to-day functioning ability, college dropout and marital problems (Agorastos *et al*., 2011).

7.4 DISCUSSION ON MAJOR FINDINGS OF PATIENTS PLASMA LEVELS FOR HALOPERIDOL AND TRAMADOL USING HPLC

Several bio-analytical methods were reported for the estimation of haloperidol and tramadol in human plasma using HPLC, but the working range of some methods in the literature (Titier *et al*., 2003; Kirchherr *et al*., 2006; Sanchez de la Torre *et al*., 2005) was narrow, being 1-30 ng/ml. The adapted/ revised method as implemented in this study method covered the range 1-200 ng/ ml. This working range is justified since the reported range in literature as well as in our study confirmed the need for a wider working range (Titier *et al*., 2003; Kirchherr *et al*., 2006; Sanchez de la Torre *et al*., 2005). The current method used a simple liquid-liquid extraction procedure, unlike many methods which reported use of solid cartridges which increased the cost of analysis (Titier *et al*., 2003). The present method has a lower limit of quantification of 4.50 ng/ ml for haloperidol and 3.82 for ng/ ml for tramadol, respectively. Many reported methods have much higher lower limits of quantification (Kirchherr *et al*., 202006; Titier *et al*., 2002; Bianchetti *et al*., 1978) which may not be suitable for studies whereas in this study lower blood samples are collected. Many reported methods used higher volume (1-2 ml) for sample processing (Arinobu *et al*., 2002; Yasui-Farukori *et al*., 2004; Balant-Gorgia *et al*., 1984). The present method used a sample processing volume of 300 µL which will help in optimal use of human blood samples in clinical studies where some amount of samples have to be archived after analysis for future reference. Thus the developed method has relative advantages when compared to previously reported methods. The therapeutic range of clinical significance found to be 1.44±0.94 ng/mL within which most of the enrolled patients showed maximum clinical benefit and further haloperidol plasma levels that substantially exceeded 73.33±1.07 ng/ml may be counter-therapeutic, as evident from adverse effects observed in outliers as well as repeated doses because of the severity of illness (Mercolini *et al*., 2007) as presented in the current study (Table/ Figure). Tramadol therapeutic range of clinical significance was found to be 6.02±3.98 ng/mL with outliers exceeding 69.37±17.38 ng/ml. In particular, an increased dose beyond this level is not efficacious for patients who have not responded to lower doses.

The HPLC method for haloperidol assessment was successfully developed and validated for the detection of haloperidol in the mobile phase. However, due to the possible presence of urea and/or creatinine in urine that elutes very closely with haloperidol, the method was found not to be suitable

for the detection of haloperidol in urine. It is recommended that a method be established that elutes haloperidol at least 0.4 minutes away from the urine contents/ eluents be validated based on findings in this study.

Creatinine is an excellent indicator for the assessment of renal function and is routinely used as a simple surrogate marker for glomerular filtration rate in patients and in animal models (Kasiske *et al*., 1996). In this study it was decided not to go further with the estimation of haloperidol and tramadol in patient's urine, due to chromatography overlap. This is due to the fact that most of the patients were managed for acute kidney injury with high levels of urea and creatinine in their plasma. A simplified method for HPLC determination of creatinine in mouse serum found that picric acid assay can overestimate serum creatinine up to six-fold over the HPLC assay in patients with low creatinine level (Yuen *et al*., 2004). The same study also indicated effectiveness of treatments for impaired renal function, which can result in underestimation of drug treatment effectiveness (Yuen *et al*. 2004).

Compared to the current method used in this study, an improved method that also offers good accuracy and similar precision with plasma samples was validated by Johns *et al*. (2001). They found it to be easily scalable to the simplest form of isocratic HPLC with a single channel UV detector. The method also offers excellent reproducibility of the results over time and between different types of columns. It also offers the potential for good laboratory practice validation based on one or two point calibration (Johns *et al*., 2001).

Determination of haloperidol and tramadol in urine using HPLC and our method proved to be difficult in this study, due to overlapping peaks assessed to be urea and creatinine found in urine. Another reason is the peaks co-eluting with the other drugs/drug metabolites, or alternatively, there might be degradation of the samples as a result of a long period of storage which in the case of this study was over three years.

7.5 LIMITATIONS

There are some limitations in this study which warrant attention:

The determination of cortisol can be performed in plasma as well as in serum. This kit has been validated for use with citrate and heparin plasma, but not tested with EDTA plasma. The blood collected in this study was done in the normal tubes used for daily diagnostics namely EDTA tubes, which could have affected the sensitivity of the concentration of cortisol in the patients' plasma.

Meanwhile, some drugs may interfere with the steroids receptors thereby reducing the levels of these hormones.

Long time storage of urine samples may degrade leading to loss of bioactive ingredients over time whereby affecting outcome.

Over sedation may mimic sleep both in pattern and quality; this may affect the report of patient's sleep.

7.6 RECOMMENDATIONS

Recommendations arising from the study include:

Delirium: patients that are placed on daily sedation during the acute phase of their management, going forward should be delayed for quality sleep assessment until patient stabilizes; this is because sedation resembles sleep both in pattern and quality.

HPLC: We recommend the use of solid phase extraction technique to remove any contaminant urine before quantifying drugs in order to allow concentration and purifying of drugs before detection.

Spectrophotometry/ ELIZA: At least 2-4 samples of blood at different time interval that coincides with the hormones peak should be considered, for better accuracy in determining hormone levels.

Sleep: Pain management should be considered as priority in the ICU, because most of the patients reported pain as the main factor that deter their sleep in ICU

Other antipsychotics: Newer generation antipsychotics like risperidone, quetiapine and olanzapine should also be evaluated for their effect on stress hormones considering the fact that they are atypical/ newer generation compared to typical antipsychotic like haloperidol

ICU environment: Dim light should be considered as against the traditional white light in the ICU environment this we belied will improved the circadian rhythm of the patients and ultimately improve their sleep quality.

7.7 FURTHER RESEARCH

The study was conducted in a single general ICU setting to access the effect of haloperidol on stress hormones and quality sleep among critically ICU patients. Given the complexity of the general ICU and the associated challenges, further studies should be conducted in other specialized ICUs in order to explore clinical competency and other assessment tools.

Finally, the implications for clinical research among ICU patients are significant. Further studies should focus on clinical intervention research among these patients with sole aim of promoting their well-being and recovery of patients with critical illness.

7.8 CONCLUSION

The aim of the study, to determine the effect of haloperidol on stress hormones and effect of quality sleep among ICU patients with delirium has been met. The ICU nurses record on sleep quality of patients can be reliably used to report patient's sleep. However, patients on antipsychotics demonstrated poor sleep quality compared to those that are not on it. Critically ill ICU patients showed high level of anxiety after ICU discharge. The study reported pain as the main cause of sleep disturbance among these patients.

The method developed was simple, specific, linear, accurate and precise and can be used for plasma haloperidol/ tramadol in routine clinical practice. The method also utilized a small processing blood sample with LLOD/ LLOQ in addition to short runs time. Acute kidney injury was the most frequent diagnosis among the critically ill ICU patients which was either caused by acute poisoning or sepsis respectively. Acute kidney injury was found to be the major cause of death among these patients. The SAPS II score predicted mortality was 30.8% compared to precise mortality of 14% among critically ill ICU patients.

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Appendix- A **SEVERITY OF ILLNESS ON ADMISSION TO INTENSIVE CARE UNIT (SAPS II)**

Variable / Scoring Guidelines		Findings	Points	Score
2.1	**Age in Years** *age in years at time of last birthday*	**< 40** **40 - 59** **60 - 69** **70 - 74** **75 - 79** **> = 80**	**0** **7** **12** **15** **16** **18**	
2.2	**Heart Rate in beats per minute** *use the highest or lowest heart rate in past 24 hours whichever gives the higher number of points*	**< 40** **40 - 69** **70 - 119** **120 - 159** **> = 160**	**11** **2** **0** **4** **7**	
2.3	**Systolic Blood Pressure in mmHg** *use the highest or lowest blood pressure in past 24 hours whichever gives the highest number of points*	**< 70** **70 - 99** **100 - 199** **> = 200**	**13** **5** **0** **2**	
2.4	**Body temperature** *use highest temperature*	**< 39 C** **> = 39 C**	**0** **3**	
2.5	**If on ventilation or CPAP PaO2 / FiO2** *use only if on ventilation or CPAP using the lowest ratio*	**< 100** **100 - 199** **> = 200**	**11** **9** **6**	
2.6	**Urinary Output in L per 24 hours** *if time period less than 24 hours adjust urine output for period to 24 hours*	**< 0.500** **0.500 - 0.999** **> = 1.000**	**11** **4** **0**	
2.7	**Serum Urea mmol/L** *use the highest value*	**< 10** **10 - 29.9** **> 30**	**0** **6** **10**	
2.8	**WBC count in 1000 per µL** *use the highest or lowest WBC in past 24 hours whichever gives the higher number of points*	**< 1.0** **1.0 - 19.9** **> = 20**	**12** **0** **3**	
2.9	**Serum Potassium in mmol/L** *use the highest or lowest potassium in past 24 hours whichever gives the higher number of points*	**< 3.0** **3.0 - 4.9** **> = 5.0**	**3** **0** **3**	
2.10	**Serum Sodium in mmol/L** *use the highest or lowest sodium in past 24 hours whichever gives the higher number of points*	**< 125** **125 - 144** **> = 145**	**5** **0** **1**	

2.11	**Serum Bicarbonate in mmol/L** *use the lowest value*	**< 15** **15 - 19** **> 20**	**6** **3** **0**	
2.12	**Serum Bilirubin in µmol/L** *use the highest value*	**< 4.0** **4.0 - 5.9** **> = 6.0**	**0** **4** **9**	
2.13	**Glasgow Coma Scale** *use the lowest value if patient sedated* *use the score before sedated*	**< 6** **6 - 8** **9 - 10** **11 - 13** **14 - 15**	**26** **13** **7** **5** **0**	
2.14	**Chronic Diseases** *HIV positive with AIDS defining opportunistic infection or tumor; malignant lymphoma Hodgkin's disease leukaemia or multiple myeloma; metastases demonstrated at surgery, radiographically or other suitable method*	**none** **metastic carcinoma** **hematologic malignancy** **AIDS**	**0** **9** **10** **17**	
2.15	**Type of admission** *scheduled surgery if scheduled at least 24h prior to operation; unscheduled if operated on with less than 24h notice; medical if no surgery within 1 week of admission to ICU*	**scheduled surgery** **medical** **unscheduled surgery**	**0** **6** **8**	

SAPS II Score	

APPENDIX- B: Evaluation form for Depression Anxiety Stress Scales (DASS)

DEPRESSION ANXIETY STRESS SCALES (DASS)

Instructions:

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you over the past week. There are not right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of the time
3 Applied to me very much, or most of the time

1	I found it hard to wind down	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feelings at all	0	1	2	3
4	I experienced breathing difficulty (eg excessively rapid breathing, breathlessness in the absence of physical fatigue	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (eg in the hands)	0	1	2	3
8	I felt I was using a lot of nervous energy	0	1	2	3
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15	I felt I was close to panic	0	1	2	3
16	I was unable to become enthusiastic about everything	0	1	2	3
17	I felt I wasn’t worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I was aware of the action of my heart in the absence of physical exertion (eg sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life was meaningless	0	1	2	3

APPENDIX C: POST TRAUMATIC STRESS (PTSD)

POST TRAUMATIC STRESS CHECKLIST

Have you experienced the problem over the last days?

Item	Statements	Not often	Rarely	Some-times	Often
1	Had upsetting thoughts or images about your time in ICU?				
2	Experienced “flashbacks” when you are reminded of your time in ICU?				
3	Felt upset when you are reminded of your time in ICU?				
4	Had bad dreams or nightmares about your time in ICU?				
5	Felt anxious or unwell when reminded of your stay in ICU?				
6	Tried not to think about, talk about, or have feelings about your time in ICU?				
7	Avoid activities, people or places that remind you of the ICU				
	Scoring				

APPENDIX D: METHODOLOGY CHART-FLOW

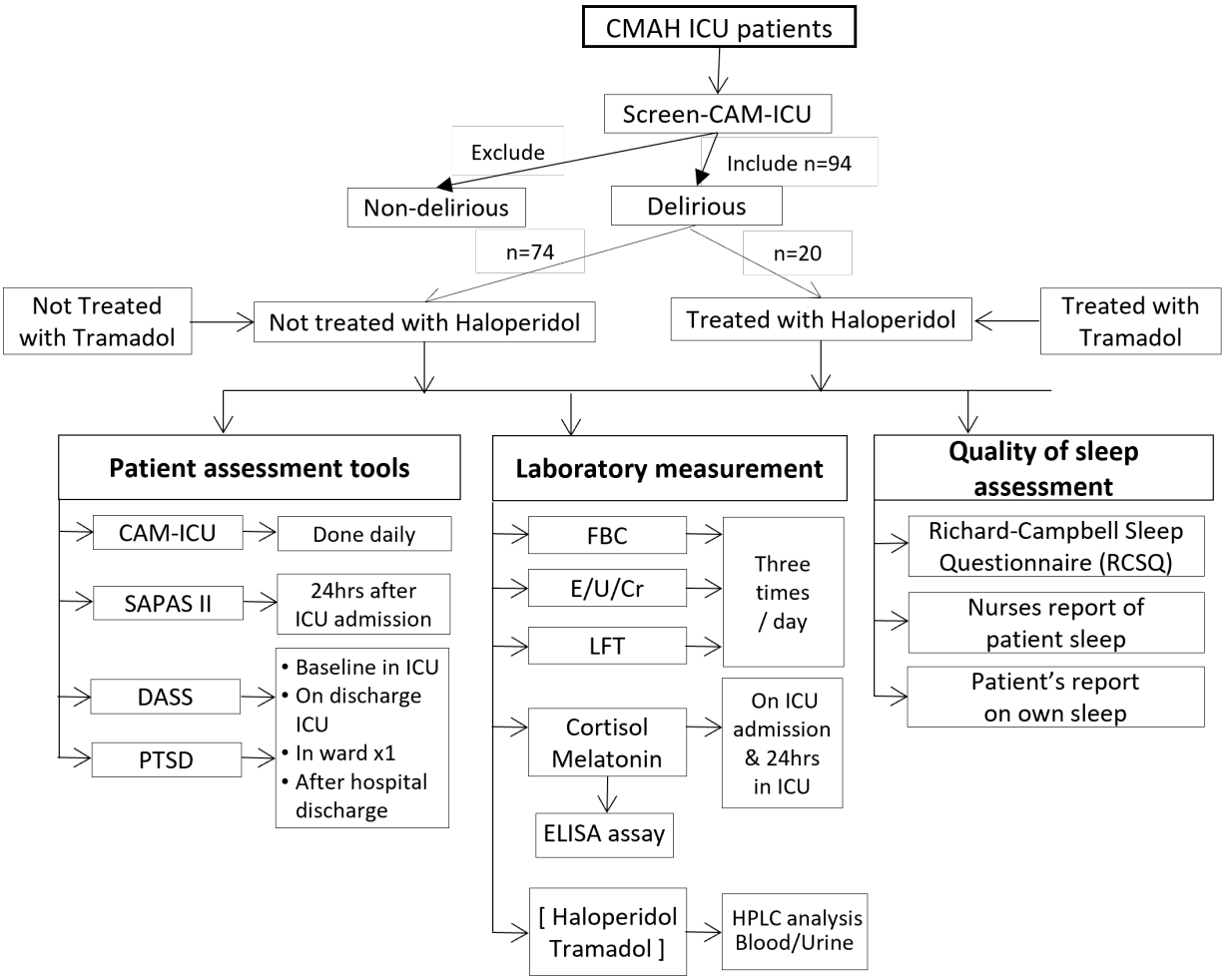


Figure D.1: Flow diagram of project design and methodology.

APPENDIX E: RICHARD-CAMPBELL SLEEP QUESTIONNAIRE (RCSQ)

1. Demographic characterization

Patient study number…….. Patient hospital number……

Age (<20…….) (20-30……) (31-40……) (41-50……) (51-60……) (61-70……) (>70……)

Gender (Male……..) (Female.....)

Race: (Black……..) (White……) (Indian……..) (Coloured……) (Asian .....)

Date of admission…….. Duration of admission…… SAPS

score……Sedative drugs used…… Patient

diagnosis……

Measure	Question
1. Sleep depth	My sleep last night was: light sleep (0)…….. deep sleep (100)……..
2. Sleep latency	Last night, the first time I got to sleep, I: Just never could fall asleep (0) ..... fall asleep almost immediately (100)…….
3. Awakenings	Last night, I was: awake all night long (0)…… awake very little (100)……..
4. Returning to sleep	Last night, when I woke up or was awakened, I: couldn't get back to sleep (0)…… Got back to sleep immediately (100)…… ….
5. Sleep quality	I would describe my sleep last night as: a bad night sleeps (0)…….. A good night's sleep (100)……..
6. Noise/ light	I would describe the noise level last night as: very noisy (0)……. very quiet (100)……

[1]Each question is scored by using a 100 mm visual analog scale in which a higher score is better.

[2]Question 6 is not a part of the original 5-item RCSQ, but was included in this project for consistency with other studies that used the RCSQ

APPENDIX F: FACTORS THAT PROMOTE OR DETER SLEEP

1. Ward assessment of patient's sleep in ICU

Patient study number…….. Patient hospital number……

Age (<20…….) (20-30……) (31-40……) (41-50……) (51-60……) (61-70……) (>70……)

Gender (Male……..) (Female.....)

Height (kg) …… Weight (m) ……..

Race: (Black……..) (White……) (Indian……..) (Coloured…….) (Asian .....)

Date of admission…….. Duration of admission …….. APACHE score……Sedative drugs used …… Patient diagnosis ……

Verbal / writing / actions

1. "What measures or intensions assist you in falling asleep last night?

   Answer …….

2. "What activities wake you up or kept you aroused last night?

   Answer …….

APPENDIX G: PATIENT DATA COLLECTION SHEET

1. Demographic characterization

Age (<20…….) (20-30……) (31-40…….) (41-50…….) (51-60……) (61-70…….) (>70 .....)

Gender (Male……..) (Female.....)

Height (m) …… Weight (kg) ……. Patient Study No ……..

Race: (Black……..) (White……) (Indian……..) (Coloured…….) (Asian.....)

Date of admission……. Date of discharge……..

2. Admission categories

Diabetes mellitus

Malignancy

Mechanical Ventilation

Sepsis

SAPS 11 (Severity of illness)

CAM-ICU (Delirium score)

DASS (Depression Anxiety Stress)

PTSD Score

3. Clinical Outcomes

ICU non-survivors

Duration of ICU stay

4. Diagnostic admission category

Scheduled surgery

Unscheduled

Medical

5. Length of stay in ICU

1-6 Days ……

7-10 Days……

>11 Days ……..

6. Lab variables to be collected from ICU sheet

Aldosterone	High	Low	Normal

FBC- WBC-	High	Low	Normal

Platelet- High Low Normal

PCV/HB High Low Normal

E/U/Cr

Na+- High Low Normal

K+- High Low Normal

Cr- High Low Normal

Urea- High Low Normal

HIV Infection +VE -VE

AIDS: CD4+ High Low Normal

LFT:

ALT High Low Normal

AST High Low Normal

Total Albumin High Low Normal

Total bilirubin High Low Normal

Glucose High Low Normal

Insulin High Low Normal

7. Lipid variables:

Cholesterol High Low Normal

Triglycerides High Low Normal

Free fatty acid High Low Normal

BMI Underweight Moderate weight Over weight Morbid

DHEA-S High Low Normal

Total cortisol Morning Afternoon

Urine cortisol Morning Afternoon

Albumin level High Low Normal

8. Pituitary adrenal hormones

Cortisol Morning Afternoon

Growth hormone (GH)	High	Normal	High

Prolactin	High	Normal	High

ACTH	High	Low	Normal

Melatonin	Morning	Afternoon

9. Catecholamine

Adrenaline

Noradrenaline

10. Medications

Prior-medications (with dose)

……

……

…… Current medications (with dose)

……

……

……

APPENDIX H: ETHICS APPROVAL FROM WITS HUMAN ETHICS COMMITTEE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Prof Robyn van Zyl et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180747

NAME: (Principal Investigator)	Prof Robyn van Zyl et al
DEPARTMENT:	Pharmacy and Pharmacology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	The pituitary adrenal hormonal response in ICU patients with acute stress
DATE CONSIDERED:	27/07/2018
DECISION:	Approved Unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	07/11/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **July** and will therefore be due in the month of **July** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

15/11/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX I: LETTERS OF PERMISSION TO CONDUCT STUDY IN CMAH AND IN 576 MULTIDISCIPLINARY ICU

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Ms. N. Mzila
Office of the Clinical Director
Tell: (011): 488-4812
Email: Nolwazi.Mzila@gautemg.gov.za
14 November 2018

GP_201809_001

Professors Robyn van Zyl and Shelley Schmollgruber

STUDY TITTLE: The Pituitary–Adrenal Hormonal Response in ICU Patients with Acute Stress

Permission is granted for you to conduct the above recruitment activities as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic Hospital will not anyway incur or inherit costs as result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the HOD and Unit Manager or sister in charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the results of your study on completion of the research.

Supported / ~~not supported~~

Dr. M.I. Mofokeng
Clinical Director
DATE: 15/11/2018

Approved/~~not approved~~

Ms. G. Bogoshi
Chief Executive Officer
Date: 16.11.2018

GAUTENG
PROVINCIAL GOVERNMENT

DEPARTMENT OF HEALTH

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

17/10/18

To whom it may concern.
I am happy that the research project, "The pituitary adrenal hormonal response in ICU patients with acute stress" be conducted in 576 multidisciplinary ICU at CMJAH.
Yours,

GA Richards

MBBCh PhD FCP (SA) FRCP FCCP

Academic Head Division of Critical Care University of the Witwatersrand

Director Critical Care Charlotte Maxeke Johannesburg Academic Hospital and

Chief Physician Departments Medicine and Pulmonology University of the Witwatersrand

APPENDIX J: INFORMATION LETTER - RELATIVE

INFORMATION LETTER – RELATIVES

Dear ..... (Name of patient)

My name is Dr Murtala Muhammad Dandare, am a PhD student (Pharmacology) at the University of the Witwatersrand. I hope to conduct a study project and will like to request your permission to consent including your relative in my sample of patients; I hope to study while they are on admission in the intensive care unit.

The aim of the study is to flow the trends of pituitary-adrenal hormone activities among critically ill patients. By agreeing to consent in this study means that I can access your ICU records from time of admission to discharge. I will like also to ask you to allow me to use the blood that has been drawn from you by the health care personnel for your routine ICU investigations to measure your illness related level of stress while on admission. The personnel normally discard this blood once the routine ICU investigations were completed. In order to get all the required stress hormone levels, may I request if I can take a small amount of blood from you to place into a tube so that it can be measured for other stress hormone that are not routinely measured in the ICU. This blood sample will be taken along with the other routine blood samples, so no need to worry about the unnecessary discomfort.

Participation in this study is purely voluntary, and even after the study have commence you can decide to terminate your participation at any point, and there will be no consequences with regards to services you and your relative may receive from the hospital or medical personnel.

Even though you may have signed consent on behalf of the patient, your relative will be contacted after discharge from the ICU to give their permission for their information to be included in the study.

The purpose of this study is to examine the effect of stress in ICU patients as a means to obtained better understanding of the effect of critical illness on your body. No reports of this study will identify you or your relatives in any ways. In case you need a copy of the final result of the study, provide your postal details and it would be provided.

If you understand what the research is about, and are happy and willing to take part in this research, please sign the attached consent form.

Thank you for taking your precious time to read this and for taking part in research that will benefit patients in a similar situation as find yourself now, in the near feature.

Thank you Yours faithfully,

Dr Murtala Muhammad Dandare
PhD student, Department of Pharmacy and Pharmacology
+27603231645, 1332308@students.wits.ac.za

APPENDIX K: INFORMATION LETTER – PATIENT

INFORMATION LETTER - PATIENTS

Dear.....(Name of patient)

My name is Dr Murtala Muhammad Dandare and I am currently a PhD (Pharmacology) candidate with the University of the Witwatersrand. As part of the requirement for the programme, I am expected to conduct both clinical and experimental research under supervision of Professor Roby Lynne van Zyl and Associate Professor Shelly Schmollgruber. I am investigating sleep perception and the pituitary-adrenal-hormonal response in ICU patients with acute stress. I would be very grateful if you would agree to participate in this study project. You are selected as potential participant been you on admission in the ICU as the research is going to be conducted in the same unit, as your management, response to treatment, personal experience, feelings and thought are of major importance to me as a researcher, and the completion of this research study.

Reason for doing research

It is a well-known fact that sleep is indispensable for the maintenance of good health, and that the need for sleep increases with illness. However, critical illness is characterized by increasing levels of pituitary stress hormones, cortisol. Critical illness is often accompanied by hypercortisolaemia that is proportional to the severity of the illness, which is attributed to stress induce activation of the hypothalamic-pituitary-adrenal axis.

What is expected of you if you choose to participate?

You would be expected to provide an answer to a total of 5 questions plus additional question about noise. Two questions regarding the activities or interventions which promote or deter your sleep will also require your response. Nurse's documentation of your sleep will also be recorded. It would be better if you answered the questions immediately based on the scale of numbers from 0 to 100, with higher score representing better sleep. The question session would approximately last for 5 to10 minutes from start to finish and would be hold in the ICU. Part of blood samples taking from you by the nurses will be used to determine some variables in the laboratory.

Benefits or risks involved in participation

By truly and faithfully stating how you feel and your experience during admission in the ICU, it may not benefit you directly but may help other subsequent patients that may experience sleep deprivation and critical illness and receive treatment in ICU in near feature. Through results and recommendations that would be published at the end of the research the management would be made aware about how you feel and correct treatment plan can be implemented so that patient with similar problem like yours would receive appropriate inpatient care and rehabilitation in near future. You may equally contact me if you require debriefing on 0603231645.

Voluntary and confidential participation

As a potential participant you have a choice as to whether participate in this research or not. There would be no consequences should you change your mind to take part in this research. If you initially agree to take part, you may withdraw at any point without having to give a reason. Your name will not appear on any of the research forms, or in the final research report. Each form will be assigned an identifiable number. I will keep a record of names in a notebook in case of a need for follow up. This book shall be seen only by the researcher and his supervisors and will be kept in a locked drawer at all times when not being used.

If you have any questions or queries at all, I would be pleased to answer them. My contact details are: Dr Murtala Muhammad Dandare. 0603231645. 1332308students@wits.ac.za

In case you need a copy of the final report, provide your postal details and it would be provided.

If you understand what the research is about, and are happy and willing to take part in this research, please sign the attached consent form.

Thank you for taking your precious time to read this and for taking part in research that will benefit patients in a similar situation as find yourself now, in the near feature.

Thank you

Yours faithfully,

Dr Murtala Muhammad Dandare

PhD student, Pharmacology Division, Department of Pharmacy and Pharmacology

+27603231645, 1332308@students.wits.ac.za

APPENDIX L: RETROSPECTIVE CONSENT FORM

RETROSPECTIVE PATIENT CONSENT FORM

I,..... (Name of the patient) understand that my relative,

…..... (name of relative) of the patient give

permission to my being included in the study and hereby gives consent for the information to be used in the study.

I have read with understanding the content of the information sheet and I have been given the opportunity to ask questions I might have regarding the procedure and my consent to my being included in the study.

Date ----------

Signature ----------

APPENDIX M: FAMILY MEMBER/ RELATIVE CONSENT FORM

FAMILY MEMBER/RELATIVE CONSENT FORM

I, _____(name) the _____ (relationship) of the patient give permission to be included in the study.

I have read with understanding the content of the information sheet and I have been given the opportunity to ask questions I might have regarding the procedure and my consent to my being included in the study.

_____ _____

Date Signature