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# Rise and fall; cortisol levels pre and post antidepressant therapy in depression

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# Rise and Fall; Cortisol levels Pre and Post Antidepressant therapy in Depression

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Psychiatry

Johannesburg, 2020

## Declaration

I, Priscilla Iswari Vythilingum Naidu, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Psychiatry at the University of the Witwatersrand, Johannesburg. This research report has not been submitted before for any degree or examination at this or any other University.



_____

Dr Priscilla Iswari Vythilingum Naidu

…24…day of ……11….2020…….in…Johannesburg……

# Dedication

To my family

Without whom none of my success would be possible

## Abstract

A consistent finding in psychiatry is Hypothalamic-Pituitary-Adrenal (HPA) axis activation in major depression. These findings would usually be expressed as hypercortisolism at baseline (Alheira, 2005; Mackin, 2004; Manthey, 2011; Young, 2004). However, there are contradicting studies illustrating hypocortisolism secondary to chronic stress or depression as a result of fatiguing of the HPA axis (Yehuda, 1995; Oldehinkel, 2001).

Major depressive disorder is a significant burden in South Africa, with a lifetime prevalence of 9.7 % (Tomlinson, 2009). Thus, it is important to understand the pathophysiology exhibited in a South African population group, in order to better the management provided.

AIM:

This study aimed to assess the severity of depression using salivary cortisol levels, as well as the Hamilton Depression Rating Scale (HAMD), both pre and post antidepressant therapy.

METHOD:

This was a prospective and quantitative study, looking at the change in cortisol levels using salivary cortisol samples and Hamilton Depression Rating Scale (HAMD) scores, before and after one month of antidepressant therapy, in a South African group of treatment naïve participants.

RESULTS:

Forty-three participants were initially recruited for this study, of which only 28 had complete data sets at the end of the study. The majority of the sample population consisted of black (89.3%), unmarried (85.7%) and unemployed (61.7%) females (92.9%), primarily in the 18-34 year old age group (60.7%) who had attained a secondary school level of education (85.7%).

Prior to antidepressant therapy, majority of the participants exhibited elevated HAMD scores (median HAMD score of 27.5; range: 16-38) and morning hypocortisolism ( median cortisol level of 0.80 nmol/L; range: 0.03-5.21); with a significant negative correlation between HAMD scores and cortisol levels at baseline. After administration of antidepressant therapy for one month, it was found that there was no significant change in cortisol concentration (median cortisol level of 0.80 nmol/L; range: 0.10-7.59). However, there was a significant decrease in HAMD score (median HAMD score of 18.5; range: 8-37). The reduction in HAMD score was <50%, thus implying considerable residual depressive symptoms. Cortisol findings were found to not be significant. There were no statistically significant difference between the change in HAMD from baseline to after one month of treatment (∆) HAMD scores and demographical data. There were also no statistically significant difference between ∆cortisol levels and demographical data.

CONCLUSION:

This study did not find high circulating cortisol due to HPA axis dysregulation amongst depressed participants, but rather hypocortisolaemia. It is postulated that, similar to other studies which reported these findings, the low cortisol levels may be attributed to chronic stress experienced by the participants as a result of their lower socioeconomic and disadvantaged backgrounds. Treatment with an antidepressant had no impact on the cortisol levels. Hence, cortisol levels in this population is not always useful as a predictor of the severity of depression or as a measure of the response to treatment as there was some improvement in the HAMD but none in the cortisol levels.

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# 1. Introduction

## 1.1. Background

*Epidemiology of depression*

Depression is one of the leading causes of morbidity and mortality in the world (Hamad, 2008). There was a global total of over 50 million years lived with disability (YLD) due to depressive disorders in 2015 (WHO, 2017). In this report, depression refers to the syndrome called unipolar depressive disorder or major depressive disorder. The diagnostic criteria include depressed mood for most of the day, loss of interest in previously pleasurable activities, significant weight loss or weight gain with decreased or increased appetite, hypersomnia or insomnia, psychomotor agitation or retardation that is witnessed by others, fatigue, feelings of worthlessness or excessive guilt, decreased ability to think or indecisiveness nearly every day, recurrent thoughts of death, and suicidal ideation or a suicide attempt (Diagnostic and Statistical Manual of Mental Disorders (DSM), 2013). The symptoms should also have been experienced for at least two weeks with no history of manic or hypomanic episodes, and the depression impacts on social and occupational functioning (DSM, 2013).

The World Health Organisation (WHO, 2017) found an increase in the prevalence of depression by 18% between 2005 and 2015, with an estimate of 322 million people globally living with depression in 2017. This has also been noted in the United States, where there was an increase in prevalence of major depression, from 3.33% to 7.06%, between 1991-1992 and 2001-2002 (Compton, 2006). In sub-Saharan Africa, neuropsychiatric disorders account for roughly 10% of the total burden of disease (Tomlinson, 2009). Focusing in on South Africa, Herman (2009) analysed data collected from 4351 adults from different South African regions and racial groups and found that the lifetime prevalence for any mental disorder is 30.3%, with one of the commonest disorders being major depressive disorder. Furthermore, this data was analysed by Tomlinson (2009) illustrating the lifetime prevalence of a major depressive disorder was 9.7% (4.9% for the past 12 months) (Tomlinson, 2009). South Africa has a higher prevalence compared to

other African countries, for example, Nigeria, with a prevalence of 3.3% (Tomlinson, 2009). Stein (2003) also found that majority of cases diagnosed at a primary care clinic in South Africa were depressive or anxiety disorders. These statistics confirm the prevalence of major depression in South Africa is high, and it correlates with that of the rest of the world (Tomlinson, 2009).

A systematic analysis for the global burden of disease study found major depressive disorder to be amongst the top ten causes of YLD in every country in 2013 (Vos, 2015). According to Bateman's article in Noseweek (2014), one-quarter of South Africans suffer from depression, leading to a 2.2% loss in annual Gross Domestic Product (GDP) (R40.6 billion) and an estimated yearly loss of earning per adult of 54 thousand Rands. Discovery Health (South Africa's most substantial medical aid) revealed a 41% increase in mental health pay-outs (from R96.7 million to R494.6 million), primarily to treat depression (Bateman, 2014). It is essential to bear in mind that 80% of the South African population is managed within the public sector and that financial statistics regarding depression, specifically, are not available (Bateman, 2014). Bateman (2014) also reports that 230 suicides are attempted daily due to depression. Depression is a significant burden on South Africa's GDP, health expenditure and social well-being. Improved and focused prevention and treatment options for those at risk of depression or diagnosed with depression is therefore vital.

*Risk factors for depression*

According to Ranga (2019), three broad factors are risk factors in the pathogenesis for depression – internalising factors, externalising factors and adversity. Some significant examples for adversity include a history of divorce, marital problems, low social support and low education. A subjective feeling of a lack of a support structure is reported to result in a two-fold increase in risk in the onset of depression (Patten, 2010).

Conversely, education appears to be a salient protective factor in preventing depression. Bauldry (2015) explained that education is protective against depression and was more significant in women compared to men, Caucasians compared to Africans and for families who had limited resources. Education is

protective as it improves economic resources, social status, quality of life and sense of worth (Bracke, 2013). Education develops one's knowledge, skills, attitudes and values and therefore aid in coping skills which in turn assist in developing and maintaining a better support network (Bracke, 2013). There is an inverse relationship/association with regards to the level of education attained and depression (Lorant, 2003). This observation has also been demonstrated in South Africa by Tomlinson (2009), where there was a higher prevalence in those with lower education (below grade 7) – they were 2.11 times more likely to develop depression in their lifetime and 3.7 times more likely to develop depression in a 12 month period.

In general, individuals have a low risk of developing depression in their early teens, with the likelihood increasing with age to peak in the 40-49 years old age group (latter age group were 1.71 times more likely to develop a depressive episode) (Tomlinson, 2009).

Depression also results from an interplay between genetics and the environment (Ranga, 2019). As altered gene expression contributes a vulnerability towards the development of depression as demonstrated in twin studies (Ranga, 2019). There is also a higher hereditability component in the development of female depression compared to male depression (Seedat, 2009). Females were 1.75 times more likely to experience lifetime depression and 2.17 times more likely to experience 12-month depressive episode compared to males (Tomlinson, 2009).

## 1.2 Cortisol dysregulation in depression

*Physiology of Cortisol*

The normal physiology of the Hypothalamic-Pituitary-Adrenal (HPA) axis is that it involves a feedback system between the hypothalamus, anterior pituitary gland and the adrenal gland. In response to circadian rhythm or stress (mental or physical), the hypothalamus secretes corticotropin-releasing hormone (CRH). CRH travels through the portal system which runs down the pituitary stalk and acts on the anterior pituitary gland to secrete a pulse of adrenocorticotropin (ACTH). A

majority of the hormones secreted by the anterior pituitary gland are under predominantly positive control by the hypothalamic releasing hormones, including ACTH. ACTH travels through the peripheral bloodstream and causes the adrenal gland, in a zone called the zona fasciculata, to secrete glucocorticoid (cortisol in humans). Cortisol then acts on the hypothalamus and pituitary gland via a negative feedback loop, and it is this vital feedback loop that leads to a decrease of CRH and ACTH and thus suppression of cortisol, as illustrated in figure 1.1 (Miller, 2007; Mackin, 2004).

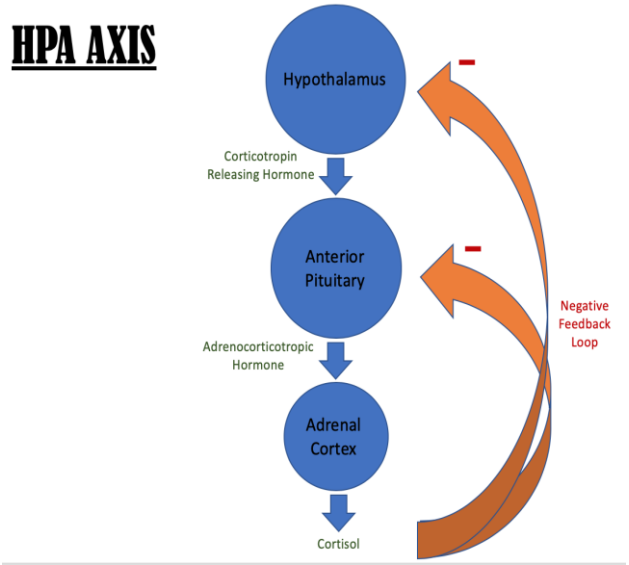



**Figure 1.1: Basic Hypothalamic-Pituitary-Adrenal axis**

The HPA axis is essential in regulating many peripheral functions such as the metabolic system (regulates glucose storage and utilisation) and immune system (involved in the inflammatory response and lymphocyte maturation) (Miller, 2007; Mackin, 2004). The HPA axis is of particular interest in the area of psychiatry as not only does it regulate the metabolic and immune system but is also able to modulate the central nervous system directly. Regulation of the HPA axis appears to be important in the pathophysiology of many psychiatric disorders. For example, glucocorticoids regulate neuronal survival, neurogenesis, the sizes of complex anatomical structures, such as the hippocampus which is essential for the acquisition of new memories and the emotional appraisal of events (Pariante, 2008).

*Cortisol and Depression*

Stress results in a significant endocrine response and activation of the HPA axis (Alheira, 2005). The activation of the HPA axis is a part of the first-line response to a stressor, the other being the autonomic nervous system ('fight or flight' response). This activation of the HPA axis alters behavioural and physiological responses, though these are not as clearly delineated as the sympathetic nervous system (McEwen, 2007). Importantly, stress hormone activation is a normal response for survival. Over time, these responses to stress can be either adaptive or damaging (Mackin, 2004).

It has been well established that a significant cause for mood disorders is the dysfunction of the endocrine system (Mackin, 2004; Manthey, 2011; Young, 2004). In the models reviewed by Miller (2007), stress leads to an increase in ACTH and circulating cortisol. With the removal of the stressor, the cortisol secretion decreases to normal. However, in some patients, there is a sustained increase in cortisol levels, which can be attributed to early life events and genetic factors (McEwen, 2007). These early life events and genetic factors cause an altered stress response pattern (prolonged HPA axis activation) to a psychological stressor in later life (McEwen, 2007). A sustained increase of cortisol results in disease by causing tissue damage and subsequent biological dysregulation such as depression (Miller, 2007). Physiologically, there have been consistent findings that 40-60% of untreated patients with major depression will present with high circulating cortisol due to HPA axis dysregulation (Parker, 2003).

There is a large body of evidence that supports HPA axis dysregulation with major depression. Pariante (2008) and Alheira (2005) showed non-suppression of the HPA axis after an oral dose of synthetic glucocorticoid dexamethasone; where the typical response should have been a potent feedback inhibition of the HPA axis. Otte (2004) illustrated HPA hyperactivity in a depressed adult group of 693 participants in the United States patients by collecting a 24-hour urine sample and testing for cortisol– patients with the highest levels of cortisol (≥47.4µg/day) had two-fold increased odds for having depression. Otte (2004) also found that increased cortisol levels were proportional to the severity of the depression (patients with severe depression had 42±28µg/day versus patients with no

depression 36±19µg/day). Overall, depressed patients had higher 24-hour urinary free cortisol than patients with no depression. According to Young (2004), there was HPA axis activation in a group of 20 depressed premenopausal women, recruited from United States' colleges. HPA axis activation was demonstrated as the group had increased pituitary secretion (ACTH) during the metyrapone (inhibits cortisol synthesis) challenge. Another study showed increased cortisol levels post dexamethasone suppression test in patients with major depressive disorder (1.13 standardised mean difference from control patients) (Miller, 2007). Other studies found enlarged pituitary and adrenal glands in patients with major depression, suggesting sustained increased circulating cortisol (Mackin, 2004).

The corticosteroid receptor hypothesis of depression suggests that there is an impaired corticosteroid receptor signalling (Herr, 2003). The theory suggests that there is impaired corticosteroid receptor signalling which is one proposed mechanism for the pathogenesis of depression. The reason that this occurs is the glucocorticoid receptor (GR) is the receptor, found in the hippocampus, hypothalamus and the pituitary gland, that cortisol binds to in order to activate the HPA axis negative feedback loop. If there is impaired receptor signalling, it causes HPA axis hyperactivity, which results in increased release of CRH, ACTH and cortisol (as their secretion is not inhibited), which, ultimately, leads to depressive symptoms (Herr, 2003).

McEwen (2007), also described that there are receptors for adrenal steroids (cortisol) in the hippocampal formation, which is an essential area for spatial, episodic and contextual memory formation. The hippocampus also plays a role in the decrease of HPA axis activation. Therefore, if there was a loss of hippocampal size/function, then there is a prolonged HPA axis stress response to psychological stressors. McEwen (2007) was able to demonstrate a reduced hippocampal size in a group of people over five years who had raised cortisol with every exam. Besides a prolonged stress response, there was also impaired hippocampal related function. Such hippocampal impairment included hippocampal-dependent memory tasks, which if extrapolated could demonstrate that those with depression could have affected memory formation. This is further corelated by Zhou (2017), who after reviewing 10 case control studies with 299 depressed participants

ranging from Asia, Europe, Australia and United States, found cognitive impairment in those with Major depression. These findings support the impaired concentration symptom as described in the criteria in the DSM 5 for major depressive disorder (DSM, 2013).

Other symptoms described in the DSM 5 include changes in sleep pattern (DSM, 2013). Inadequate or poor sleep could be as a result of elevated cortisol, as cortisol causes an increase in sympathetic nervous system activity and decreased parasympathetic nervous system activity (McEwen, 2007). Resultant sleep impairment compounds the cognitive impairment that results from depression.

However, contradicting evidence exists, and some studies have found that those with chronic stress had reduced cortisol output. This finding has been demonstrated in studies conducted by Yehuda (1995) and Heim (2000). These studies describe cortisol secretion in response to stress as cortisol helps one maintain homeostasis under conditions of stress. However, hypocortisolism has been found in healthy individuals living under conditions of ongoing stress (Oldehinkel, 2001). Alterations at several levels of the HPA axis from genetics, gender or early life stressors may predispose one to hypocortisolism. It is vital to hold in mind that there are many variables amongst different population groups which can complicate the scenario. Some groups studied are Vietnam veterans and Holocaust victims; they had decreased 24-hour urinary cortisol compared to the general population (Yehuda, 1995). These groups had a higher glucocorticoid receptor number in the brain, which was hypothesised to have increased sensitivity to the HPA axis negative feedback loop inhibition resulting in increased cortisol suppression. In those with multiple co-morbid psychiatric conditions, it can become complicated. As Yehuda, (1996) showed that those with Post Traumatic Stress Disorder (PTSD) had lower basal cortisol levels, and cortisol secretion was closer to the normal circadian rhythm with regards to secretion. However, patients with depression had more chaotic cortisol secretion patterns (less rhythmic) showing HPA axis dysregulation and higher circulating cortisol (Yehuda, 1993).

*South African studies*

Similar to international studies, South African studies have found mixed results concerning cortisol dysfunction in depression. According to De Villiers (1987), their findings revealed that patients diagnosed with a major depressive disorder had significantly higher cortisol plasma concentrations compared to the controls. The depressed group were subdivided into three groups - sporadic depressive disease, familial pure depressive disease and depression spectrum disease groups. All these groups displayed higher cortisol levels compared to the control group, with the patients in the sporadic depressive group showing the highest levels of plasma cortisol, followed by the depression spectrum group. They attributed the increased plasma cortisol levels to hyperactivity of the sympathetic nervous system and the HPA axis dysfunction in depression.

In contrast, Malan (2017) found that exposure to chronic psychological stress leads to a dysregulation of the HPA axis, resulting in downregulation or hypo-responsiveness of cortisol. They describe chronic psychological stress as chronic depressive symptoms arising from mental stress. Where lower levels of circulating cortisol were found in black males as well as more depressive symptoms compared to white males. They suggested the possible HPA axis exhaustion was due to chronic emotional distress, with desensitised cortisol. Hypocortisolaemia as a result of HPA axis dysfunction was also found in a study by De Kock (2015) in African patients reporting severe stress. Interestingly, a study by Mashele (2014) did not demonstrate any direct association between depression and circulating cortisol; but they did note blunted neuroendocrine responses linked to depressive symptoms.

In summary, there are many inconsistencies that exist with the literature with regards to a correlation between circulating plasma cortisol levels and depression. Some studies describe hypercortisolism, while others describe hypocortisolism. It appears that childhood trauma and chronicity of the depression are significant possible factors for the difference in cortisol levels. Thus, certain societal groups may have an increased likelihood of developing either hyper or hypocortisolaemia.

## 1.3. Measuring cortisol levels

A standard method of measuring HPA axis activity, is the measurement of cortisol output. Cortisol can be measured through various substrates such as blood, hair, saliva and urine, and each substrate would contain different concentrations of cortisol (Seth, 2016; Otte, 2004; Manthey, 2011). Different substrates have different concentrations, as some would test cortisol that is bound to a cortisol-binding globulin/protein, and others would test free cortisol. Overall, each substrate has strengths and limitations when measuring cortisol. Different methodologies are used depending on the clinical or research objective; for example, single cortisol sampling would be used to correlate physiological/affective characteristics with the cortisol level. In contrast, multiple samplings would be used to establish a baseline and describe a trend post a stimulus (Levine, 2007).

For example, using blood substrate for cortisol measurement requires venepuncture. This procedure is costly, invasive, requires medical staff, specialised equipment and may require special handling as it may be considered a biohazard. Another drawback is the assumption that further venepuncture at later stages may elicit a cortisol response and thus give falsely elevated levels (Seth, 2016). In a clinical setting, a random total plasma cortisol sample (blood sampling) would be useful in determining any abnormal variation of the diurnal secretion of cortisol. If found, then further testing may be needed (Levine, 2007). However, total cortisol may not be diagnostically useful in determining adrenal function. In these cases, free cortisol may be a better test (hypoproteinaemia as binding globulins need to be taken into account) (Levine, 2007).

The form of cortisol found in saliva is called free cortisol which is biologically active cortisol that is unbound to carrier proteins, and it is this free cortisol that diffuses freely into the saliva and urine. Therefore, salivary and urinary cortisol measurements reflect the biologically active free cortisol rather than total cortisol (Read, 1990).

Measuring salivary cortisol has many advantages such as being stress-free, non-invasive, rapid, it allows for frequent sampling to be done, and there is no need for trained staff or specialised equipment (Levine, 2007; Haeckel, 1993). It is also convenient as sampling is not limited to a medical/laboratory setting (Levine, 2007). However, there are still some disadvantages such as poor compliance if given to do in a home setting, insufficient salivary samples, samples may be taken post eating, or drinking (affecting the cortisol concentrations), or samples may be done when a patient has an oral lesion (blood affects the cortisol concentration). Majority of these limitations can be resolved by collecting the samples in a clinical setting (Levine, 2007) as it is operator dependent (Miller, 2007; Blair, 2017). Also, to note, cortisol found in saliva would usually be lower than blood serum due to the conversion of cortisol to cortisone (an inactive metabolite) in saliva (Seth, 2016). Salivary cortisol sample reflects HPA activity for the past 10-60 minutes (Miller, 2007).

Furthermore, Seth (2016), explains that testing urine for cortisol requires a 24-hour collection of urine. Collecting a 24-hour urine sample would be essential to assess the cortisol production over 24 hours compared to a single point in time as ACTH and cortisol are secreted in discrete pulses thus having episodic periods of rising and falling of cortisol levels (Nieman, 2017a). The disadvantages of this type of collection would be the inability to identify fluctuations or variations of cortisol at specific points in the day (Seth, 2016). It is dependent on normal renal functioning, requires concurrent creatinine measurements, and there can be over or under collection of urine resulting skewed results (Nieman, 2017a) as it is patient dependent (Miller, 2007; Blair, 2017).

Dexamethasone suppression test is another method of cortisol measurement. The dexamethasone suppression test works by inhibiting the pituitary gland from secreting ACTH by activating the negative feedback loop with an exogenous steroid. If there is no cortisol suppression, then pathology exists (Miller, 2007; Alheira, 2005). This test is useful in diagnosing adrenal hyperfunction; however, it requires assessment in a medical facility, requires specialised staff and venepuncture (Lacroix, 2017).

With the above kept in mind, salivary cortisol sampling is the preferred choice for this type of study as there is a need to do the testing in a non-specialized facility, be the least invasive and have the flexibility of convenient sampling. The limitations explored in this type of sampling can be minimised by employing the researcher to direct and monitor cortisol sample collections.

### 1.3.1 Salivary collection, time and assay

Providing a salivary sample for cortisol measurement is simple in both children and adults, where they can either salivate directly into a tube or chew on a piece of cotton wool (Blair, 2017). Studies have shown that the salivary concentration was independent of flow rate and that there was no difference between whole salivary cortisol concentrations compared to parotid salivary cortisol concentrations (Read, 1990).

Storage of these salivary samples is relatively uncomplicated and gives the clinician time for analysis as samples can be kept at room temperature for three days, up to seven days if kept at 4°C, and for later measurement, it can be stored at -20°C for up to nine months (Read, 1990). A South African NHLS Memo dated 16 August 2016, describes the procedure of salivary cortisol sample collection and storage. This memo states that the collection tube containing the saliva saturated cotton wool should be sent to the laboratory after collection as it is stable at room temperature 20-25°C for 24 hours. If transport is delayed then the sample can be refrigerated as it is stable at 2-8°C for four days; however, if there is a longer delay to processing, then the sample can be stored at -20°C for twelve months.

Salivary cortisol concentrations have diurnal variation, reaching a peak around the time of awakening with a concentration of about 5.6 ng/mL (15.4 nmol/L) at 8 to 9 AM and a nadir after the onset of sleep about 1 ng/mL (2.8 nmol/L) at around 11 PM (Laudat, 1988; Levine, 2007). Therefore, collection times should be based on this diurnal variation (Laudat, 1988). This recommendation is corroborated by Seth (2016). Seth (2016) stated that cortisol concentrations are significantly increased 30-45 minutes post waking and a subsequent decline throughout the day and

reaching the nadir at around midnight. Based on the literature, the ideal time for sampling cortisol would be an early morning sample, around 30-45 minutes post-awakening.

Salivary cortisol can be measured using different forms of assays; there are competitive protein-binding assays, radio-immunoassays (RAI), or enzyme immunoassays (EIA). The test can be performed successfully by anyone with access to a plate reader, a shaker, and pipettors (Read, 1990; Nieman, 2017b; Manthey, 2011; Blair, 2017). A previous worry was the accuracy of the salivary cortisol assays; however, salivary cortisol could be substituted for plasma cortisol as it provides similar data (Galard, 1991). According to Raff (2002), EIA produced consistently higher salivary cortisol values compared to the RAI, and the RAI produced salivary cortisol results closer to the expected value of the provided cortisol stock solution diluted in saliva. For this reason, RAI would be preferred as the results would be more accurate.

### 1.3.2 Factors affecting cortisol levels

It is essential to keep in mind that many factors can affect the levels of cortisol in a person; these include age, gender, certain medications, certain medical and psychiatric conditions and shift workers.

*Age*

Cortisol levels are significantly increased in older patients and are more pronounced in the nocturnal levels of cortisol. It is hypothesised that ageing affects HPA axis functioning in both men and women (Larsson, 2009). Van Cauter (1996) corroborated increased cortisol levels in older patients; where they found that ageing was not associated with changes in basal activity of the HPA axis but that there was an age-related impairment in the cortisol feedback to inhibit the HPA axis.

*Gender*

There are gender variations in cortisol levels. Women have a significantly higher morning cortisol level compared to men, and women below 50 years of age have both higher morning cortisol level and delta-cortisol level (morning-evening cortisol) (Larsson, 2009; Smyth, 1997).

*Medication*

Medication can affect cortisol via different pathways. Firstly, medication can have direct agonistic and antagonistic effects on the HPA axis (affecting physiological systems connected with the HPA axis) (Granger, 2009). Secondly, medication can have an iatrogenic effect on the availability and composition of saliva, or lastly, alter the antibodies used in the immunoassay (Granger, 2009). Thus, medication effects may lead to salivary cortisol measurement errors. For example, synthetic glucocorticoids/exogenous glucocorticoids may falsely elevate readings or later indirectly lower readings via the HPA axis suppression (Nieman, 2017b). Another example are drugs that induce the hepatic cytochrome P-450 enzymes leading to increased metabolism of steroids (such as barbiturates, phenytoin and rifampicin) (Nieman, 2017b). Medication can also bind to albumin or glycoprotein, which then affects the free cortisol concentration (Haeckel,1993; Nedefors, 1992).

*Medical conditions*

Medical conditions that affect the cortisol binding globulin would affect the free cortisol, thus influencing cortisol measurements. Conditions affecting cortisol-binding globulin include diabetes (specifically hyperinsulinaemic states), hyperthyroidism, hepatic and renal dysfunction and sepsis (Nieman, 2017b). In another study, chronic fatigue syndrome, fibromyalgia, chronic pelvic pain, and asthma also affected cortisol resulting in hypocortisolism (Heim, 2000). Conditions that affect the HPA axis would affect cortisol secretion, such as Cushing's disease, Addison's disease and those who have rheumatoid arthritis as they may have altered diurnal variation of cortisol (Smyth, 1997).

*Illicit substance use*

Substance misuse affects the brain's motivational systems which lead to altered HPA axis function. With acute use, substances such as alcohol or nicotine can cause a stress-like cortisol response. If there is ongoing use, it causes HPA axis

dysregulation. It should be noted that post quitting a substance; there may be deficient cortisol reactivity in response to stressors (Lovallo, 2006). Ecstasy/MDMA is another example of how substance use can affect the HPA axis; MDMA increases cortisol in the acute and subacute period which affects how those patients would respond to stress (Parrott, 2014).

*Other major psychiatric illnesses*

Cortisol levels are particularly affected in women diagnosed with an anxiety disorder where the cortisol was found to be blunted. However, in males, cortisol was found to be elevated in those diagnosed with a social anxiety disorder (Zorn, 2017). For those diagnosed with schizophrenia, cortisol stress reactivity was blunted in both men and women (Zorn, 2017). Patients who have developed post-traumatic stress disorder also have altered cortisol levels; with hypocortisolism frequently being found in previously healthy individuals who are now suffering from ongoing stress (Heim, 2000; Smyth, 1997).

*Shift workers*

Cortisol levels are affected in shift workers as their circadian rhythm has been altered to accommodate the change in working hours (Laudat, 1988; Smyth, 1997; Blair, 2017).

## 1.4 Cortisol and antidepressants

As some of the neurobiology in depression is known, targeting certain specific pathologies in depression would be a more direct way of treating depression. This hypothesis could suggest a more specific target area for antidepressants. In some patients, antidepressants have helped in regulating the HPA axis by intensifying the feedback loop inhibition and thus decreasing the HPA axis hyperactivity in depressed patients (Pariante, 2008). Laakmann (2004) proved HPA axis regulation by demonstrating improvement in cortisol levels after mirtazapine (antidepressant) was initiated in a group of 12 patients with major depression. It has also been found after remission of the depression, post-treatment, that the adrenal gland volume becomes indistinguishable from the healthy controls

(Parker, 2003). Adrenal hyper-responsiveness also resolves back to normal (Parker, 2003). Different classes of antidepressants have shown mixed effects on the HPA axis with some normalising the circulating cortisol levels more compared to others (Herr, 2003).

Improvement in hypercortisolaemia was shown by Kapitany (1999), where 16 patients diagnosed with acute major depression were given citalopram 20 mg over a 2-day stimulation procedure resulting in cortisol levels dropping to a lower level compared to the placebo group (from 9.4 mg dl to 8.8 mg dl). This improvement had been reproduced in another study by Dziurkowska (2013), who illustrated that there was cortisol suppression in the saliva in 40 depressed patients who were treated with selective serotonin reuptake inhibitors. Navines (2007) also demonstrated a decrease in HPA axis overactivity in a group of patients treated with citalopram for eight weeks. These patients showed a reduction in both ACTH and cortisol after the citalopram course. Based on the literature, it appears that antidepressants are an effective treatment for hypercortisolaemia in depression as it normalises the hyperactivity of the HPA axis.

## 1.5. Rating scale in depression

Rating scales may be useful in both research and clinical practice. Rating scales assist to prove, with evidence, that the treatment option chosen was effective (Gou, 2015). Gou (2015) demonstrated a higher remission rate when clinicians up-titrated medication after reviewing standardised self-reported feedback from their patients. The benefits of rating scales were further demonstrated by Chang (2014), who found higher remission rates in those whose management plan included assessments of symptoms and feedback to the clinicians.

Another benefit for the use of rating scales is to identify non-responders and those with residual symptoms, as a lack of improvement/residual symptoms require a change in treatment to avoid relapse (Szegedi, 2009; Pintor, 2003; Faravelli, 1986). Rating scales can also illustrate improvement, which is useful in patients

who are dysphoric and pessimistic about their progress on treatment (Tadić, 2010) as this will aid in their adherence (Dowrick, 2009).

However, there are disadvantages to the use of rating scales. Patients may feel overburdened by having to complete rating scales, especially if there are many single-item questions with four or five choices per question. This burden may lead to drop out of treatment or to seek treatment elsewhere (Dowrick, 2009). Clinicians may also feel reluctant to perform rating scales in clinical practice, as they may perceive this a time burden (Cusin, 2009). Also, there are some rating scales that need to be purchased and therefore add to the financial burden on patients.

There are many different rating scales; a few examples are the Hamilton Depression Rating Scale (HAMD), Geriatric Depression Rating Scale and the Montgomery-Asberg Depression Rating Scale (MADRS). The HAMD has been the gold standard for the assessment of depression, and a great advantage is that it is also able to measure the severity of depression (Cusin, 2009). Since antidepressant use in the 1960s, the HAMD has been the most commonly used rating scale for depression with a 90% sensitivity and 63% specificity for diagnosing depression (Dura, 1990). It is the most frequently used scale in clinical trials and has been used in over 500 studies as the primary efficacy measure (Cusin, 2009). However, the HAMD was mostly used in research to discriminate between placebo and active drugs or to show a dose-response relationship in patients with major depression. (Dura,1990; Bech, 2006).

An advantage besides the continuous and widespread use of the HAMD is the improved inter-rater reliability with the use of a structured interview guide (Cusin, 2009). The internal, inter-rater and retest reliability estimates are mostly suitable for the global score but may be weaker for individual items (Bagby, 2004). The HAMD is easy to score, and the cut-offs in scoring stratify the level of severity of depression (Cusin, 2009). The HAMD is easily accessible as it is widely available in the public domain and is not protected by copyright.

Disadvantages for the HAMD is that it is time-consuming in a clinical setting, taking an average of twelve minutes to complete, which was likely underestimated

(Cusin, 2009). Other disadvantages include measuring concepts that are old and not including essential criteria from the current DSM diagnosis (Bagby, 2004).

## 1.6. Correlation between HAMD and salivary cortisol level

Maes (1986) found an elevated cortisol level post a dexamethasone suppression test in 100 depressed patients. Patients were administered 1 mg of dexamethasone the previous night, and plasma cortisol levels were measured at 8 AM, 4 PM and 11 PM the following day. The plasma cortisol levels at 4 PM and 11 PM were able to differentiate between minor and major depression; however, the results were inconclusive. They were able to stratify patients with major and mild depression based on the severity of elevation of their cortisol – if plasma cortisol levels were greater than 3.5 µ/dl at 8 AM; it was the most sensitive (56.9%) and specific (94.3%) discriminator between minor and major depression. Cortisol levels were compared to the HAMD scores, which showed a correlation to major depressive disorder.

Cubala (2016) compared 20 treatment naïve patients diagnosed with major depression to control patients. Those diagnosed with major depressive disorder had a HAMD score of >17 as well as hypercortisolaemia. A study by Yoon (2015) showed that there were significant positive correlations between plasma cortisol levels and the depression component of the HAMD and vice versa. The depression component of the HAMD was related to the plasma cortisol level (Cubala, 2014).

The above findings were not demonstrated by De Villiers (1987), who found no significant correlation between plasma cortisol levels and the Hamilton Depression Rating scale.

## 1.7 Purpose of the study

To explore depression from a South African perspective as the majority of the literature is investigated in first world countries. Due to the substantial burden of depression in South Africa, studying the demographics of the affected population, ascertaining their baseline cortisol levels and HAMD scores and assessing their response to treatment would assist in gaining a local perspective with a possibility to influence management in this vulnerable population group.

## 1.8 Hypothesis

The hypothesis is that both cortisol levels and HAMD scores at baseline would be elevated and will decrease after one month of antidepressant treatment in treatment naïve participants diagnosed with major depression.

## 1.9 Aim

This study aimed to assess the severity of depression using salivary cortisol levels, as well as the Hamilton Depression Rating Scale (HAMD), both pre and post antidepressant therapy.

## 1.10 Objectives

In a group of untreated patients with major depression, who attended both initial and follow up visit:

1. To describe the demographic characteristics of the study population
2. To describe the severity of depression at entry into the study (pre-treatment) and after one month of treatment with an antidepressant using the Hamilton Depression rating scale (HAMD).
3. To describe the change in salivary cortisol levels at entry to study and after one month of treatment with an antidepressant.

4. To determine, if any, the correlation between a change in HAMD score and a change in salivary cortisol level.

# 2. Method

## 2.1 Study design:

This study was a prospective and quantitative study.

## 2.2 Site of study:

This study was a multi-site study conducted in Soweto, Johannesburg, South Africa. Sites included Chris Hani Baragwanath Academic Hospital Psychiatry outpatient department, and certain District Mental Health Clinics, specifically Dobsonville Clinic, Zola Clinic, Lillian Ngoyi Clinic, Eldorado Park Clinic and Pimville clinic.
for

## 2.3 Participants:

All participants who met the criteria were included in the study. The criteria for inclusion into this study required participants to be:

- between the ages of 18 and 65 years old
- diagnosed with depression clinically
- be treatment naïve.

The exclusion criteria for this study were:

- participants who were diagnosed with a pre-existing co-morbid medical illness (such as hypertension, diabetes mellitus and HIV, etc.) that are known to affect the HPA axis.
- participants who were prescribed medication that is known to affect the HPA axis; such as medication for depression, anxiety, seizures, cholesterol, hypertension, gastritis. These medications include, example, selective serotonin reuptake inhibitors, selective noradrenaline reuptake inhibitors, lipid-lowering agents, antihypertensives, proton pump inhibitors, benzodiazepines, anticonvulsants and other drugs.
- participants who used any illicit substance in the past six months
- participants who had another major psychiatric illness

- participants who were shift workers (affects circadian rhythm and cortisol levels)
- participants who failed to return for the follow up appointment after one month of antidepressant therapy

## 2.4 Sample size

The primary outcome measure was the difference in salivary cortisol values from before treatment and four weeks post-treatment with an antidepressant using a parametric t-test. A Power calculation was run using G Power (Faul, 2007). Although the original power calculation was based on research with a group of twelve patients by Laakmann (2004), using a mean difference of 7nmol/l and a standard deviation of 3.5nmol/l, a probability p-value = 0.05 and an estimated power of 0.85 a total sample size of 20 was found to be required to determine statistical significance. A sample size of 20 participants was felt to be an insufficient sample size. Therefore, the sample size was adjusted by changing the effect size from 0.5 to 0.3 and using Cohen's D resulting in the new sample size requiring 51 patients.

## 2.5 Study Method:

Participants were recruited during the period January 2017 to December 2017. The study was explained to the staff (nurses and psychiatrists) at each participating facility. Participants who were diagnosed clinically with major depression and had no contraindications to treatment were informed of the study were invited to participate in the study. If they were willing to participate, then they were referred to the principal researcher on the same day as treatment initiation and asked to refrain from eating 15 minutes before the meeting.

The principal researcher then saw those participants who were willing to partake in the study. Participants were seen at the clinic where they saw their psychiatrist so as to not to increase their cost burden with further travel. At this contact, the study was explained, with the researcher expanding on the reason for the research and the research objectives. The patient information leaflet and informed consent

documents were also discussed, and any questions raised were answered. Following this, participants were assessed to determine if they met the inclusion and exclusion criteria required for participation in this study. Patients who met all inclusion criteria were invited to participate in the study and to complete the informed consent document. If a patient declined to participate in the study, they were advised to commence their antidepressant and to continue treatment as prescribed by their attending psychiatrist.

*Pre-treatment data collection:*

Once written informed consent was attained, the following information was collected and recorded in a data booklet by the principal researcher:

a) Socio-demographic data namely: study number, age, gender, ethnic group, marital status, highest level of education, and family history of mental illness.
b) The severity of the depression using the Hamilton Depression Rating Scale (HAMD). This scale was performed on each participant by the principal researcher. HAMD scores were calculated and explained to all participants.
c) Saliva was collected, with the assistance of the researcher, to determine cortisol levels and the exact time of collection was recorded.

According to Zimmerman (2013), HAMD assesses the severity of depression and evaluates the performance of the antidepressant. The HAMD contains 17 items/ questions, with each item being scored on a 3- or 5-point scale. The scoring of the HAMD is as follows: 0-7 is normal/no depression, 8-13 is mild depression, 14-18 is moderate depression, 19-22 is severe depression and ≥23 is very severe depression with a maximum score of 52. It is also a widely available scale in the public domain with no copyright.

*Collection of salivary samples:*

The salivary collection process was explained in conjunction with a written protocol detailing the procedure for the collection of saliva as discussed below. The sample was collected between 8 am -12 pm as this period had minimal variation in cortisol levels. Cortisol is maximum at 8 to 9 AM and a nadir after the onset of sleep 11 PM (Laudat, 1988; Levine, 2007). The time of collection also had to be feasible to

include patient waiting times after queuing and being assessed by their psychiatrist.

With the assistance of the researcher, the top of the salivary collection tube was opened and held to the participant's mouth. The cotton wool rod inside the tube was tipped into the open mouth of the participant; where the participant had to chew on it for about 2 minutes for the cotton wool rod to become saturated with saliva. After saturation, the participant spat the cotton wool rod back into the tube, the cap was replaced and close tightly, and the tube was placed in a Ziploc bag.

The date, time and the allocated participant study number was documented on the tube. The tube was taken to the laboratory within 24 hours. If there was any delay in getting samples to the laboratory; then the samples were refrigerated between 2-8 degrees Celsius and later taken to the lab in a cooler box with ice.

*Cortisol assay:*

The salivary collection tubes were centrifuged at 3500g for 15 minutes, the cotton wool plugs were subsequently discarded after the centrifuge, and the remaining saliva samples were frozen at −20 °C to precipitate mucins for later analysis. When the salivary assay was to be performed, the sample was thawed and centrifuged at 1500 g for 15 min. All samples were assayed together in duplicate using a commercial high-sensitivity radioimmunoassay for salivary cortisol EIA (Salimetrics LLC). The samples were tested according to the manufacturer's instructions. The lower limit of detection for the assay was 0.19 nmol/l. The intra- and inter-assay coefficients of variation (CVs) were listed as < 6%. The cortisol assays were tested in the physiology department at Witwatersrand medical school, Johannesburg with the assistance of the University of the Witwatersrand's Physiology team.

*Post-treatment data collection:*

After their one month follow up visit with their treating psychiatrist, participants were seen by the principal researcher and the following was obtained and recorded in their data booklet:

1. The severity of their current depressive symptoms as assessed with the HAMD.
2. A second salivary cortisol sample was collected as close as possible to the time that the first sample was collected.
3. Adherence to their medication was enquired upon, and patients' tablets were also counted.

HAMD scores were calculated and explained to all participants. Thereafter, the patients were advised to continue with their treatment at the clinic from where they were referred. Participants were later informed of their results telephonically once the salivary cortisol samples were analysed. All salivary cortisol samples were analysed at the same time, at the end of the study's data collection.

## 2.6 Ethical considerations

Before participants were recruited, this study was reviewed and cleared to proceed by the Human Research Ethics Committee (Clearance Certificate number: M161012).

Participants who gave written informed consent to participate in the study were allocated a unique study number to ensure anonymity. The principal researcher kept the record of the patient identifying details and study number, and any identifying data would be kept confidential by the principal researcher. Identifying data was not divulged to any other member of the research team.

## 2.7 Data Analysis

The sociodemographic characteristics of the study population were described by means of proportions and percentages and illustrated using tables for graphical representation. The difference in HAMD scores and Cortisol concentration pre- and post- antidepressant treatment were analysed using a Wilcoxon signed-rank tests. Spearman rank correlations were used to analyse the relationships between HAMD scores and Cortisol concentration, both pre- and post- antidepressant treatment. Mann-Whitney U, Wilcoxon matched paired test, and Kruskal-Wallis analyses were conducted as appropriate to analyse pre- and post- antidepressant treatment HAMD scores and Cortisol concentration and each demographic variable.

Analyses were conducted in Statistica version 7 (www.statsoft.com). All tests were two-tailed, and alpha was set at 0.05

## 3. Results

Forty-three participants were initially recruited for this study, of which only 29 attended their one-month follow-up. Of these 29 participants, one had an insufficient cortisol sample prior to antidepressant therapy. The final study population thus comprised of only 28 participants, who had complete data sets.

### 3.1 Socio-demographic characteristics of the study population

Analysis of the socio-demographic data showed that there was no statistically significant difference between the age groups ($\chi^2_3$=9.85; p=0.009). Nor was there a significant difference in the number of participants who were employed versus unemployed (n=11 vs 17) ($\chi^2_1$=1.29; p=0.257) (Table 1).

Statistically significant results found in the socio-demographic data were attributed to gender, race, relationship status, education level and family history of mental illness. The gender of the participants was significantly skewed towards females (n=26 vs 2) ($\chi^2_1$=20.57; p<0.001) (Table 1). Only two race groups (Black African and Coloured) were recorded amongst the participants, of which significantly more were Black African (n=25 vs 3) ($\chi^2_1$=17.23; p<0.001) (Table 1). Significantly more participants were unmarried (n=24) or were separated compared to participants who were married or living with a partner (n=4) ($\chi^2_1$=14.29; p<0.001). The level of education of the recruited participants showed that significantly more participants had a secondary school education (n=24) compared to primary (n=0) and tertiary (n=4) education ($\chi^2_2$=35.43; p<0.001). Significantly more participants (n=22) reported no incidence of mental illness in the family compared with those who had (n=6) ($\chi^2_1$=9.14; p=0.002) (Table 1).

**Table 3.1: Frequency distribution of the demographic characteristics of the study population.**

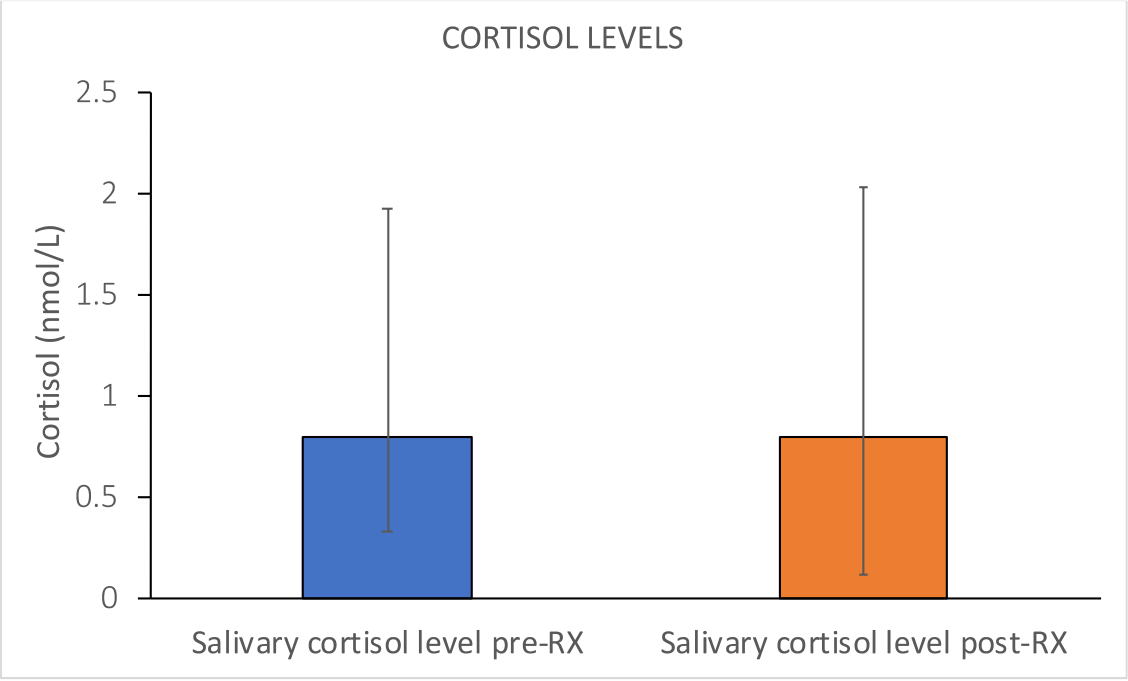
| Variables | N (n=28) | Percent | Statistics |
|---|---|---|---|
| Age Group | | | $\chi^2_3 = 9.65$; p = **0.009** |
| *18-34 years* | 17 | 60.7% | |
| *35-50 years* | 6 | 21.4% | |
| *51-65 years* | 5 | 17.9% | |
| Gender | | | $\chi^2_1 = 20.57$; **p < 0.001** |
| *Male* | 2 | 7.1% | |
| *Female* | 26 | 92.9% | |
| Race | | | $\chi^2_1 = 17.23$; **p < 0.001** |
| *Black African* | 25 | 89.3% | |
| *Coloured* | 3 | 10.7% | |
| Relationship status | | | $\chi^2_1 = 14.29$; **p < 0.001** |
| *Unmarried/separated* | 24 | 85.7% | |
| *Married/with partner* | 4 | 14.3% | |
| Level of education | | | $\chi^2_2 = 35.43$; **p < 0.001** |
| *Primary school* | 0 | 0% | |
| *Secondary school* | 24 | 85.7% | |
| *Tertiary* | 4 | 14.3% | |
| Employment status | | | $\chi^2_1 = 1.29$; p = 0.257 |
| *Employed* | 11 | 39.3% | |
| *Unemployed* | 17 | 61.7% | |
| Family mental illness | | | $\chi^2_1 = 9.14$; **p = 0.002** |
| *Yes* | 6 | 21.4% | |
| *No* | 22 | 78.6% | |

## 3.2 Cortisol levels of the study population.

Cortisol samples were collected on all 43 participants at the initial visit, prior to antidepressant therapy consumption. However, cortisol concentrations were available for only 41 participants as 2 samples were insufficient for analysis. The cortisol levels of the 41 participants at entry into study ranged from 0.032 to 6.973 nmol/L. The mean score was 1.63 nmol/L; the median was 0.83 nmol/L.

For the participants who had cortisol levels, both a pre and post antidepressant therapy (n=28), the mean cortisol level at baseline was 1.42 nmol/L (SE=0.31). The median was 0.80 nmol/L (95% confidence interval (CI) 0.82 - 2.02; range: 0.032-5.207). The mean cortisol levels after one month of treatment with an antidepressant was 1.60 nmol/L (SE=0.40). The median was 0.80 nmol/L (95% CI 0.81 - 2.34; range: 0.096-7.587) (Figure 1).

There was no statistically significant difference between the median cortisol levels at baseline and post antidepressant therapy (Wilcoxon match-pairs test: W=380.50; p=0.857)




(Error bars represent 1st and 3rd interquartile)

**Figure 3.1: Median cortisol levels concentrations of the study population.**

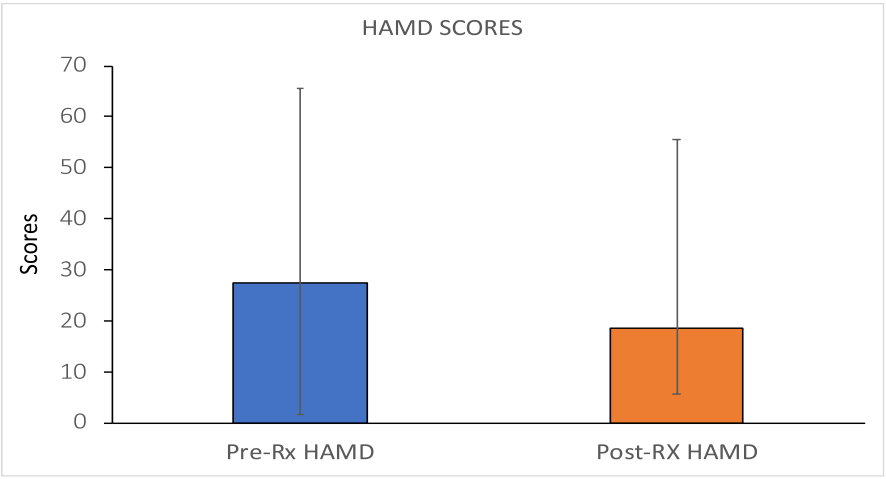
## 3.3 HAMD scores of the study population.

HAMD scores were collected on all 43 participants at the initial visit, prior to antidepressant therapy consumption. The HAMD scores ranged from 14 to 38. The mean score was 27.33; the median was 28.

For the participants who had cortisol levels, both a pre and post antidepressant therapy (n=28), the mean HAMD score at baseline was 28.36 (SE=0.92). The median HAMD score was 27.5 (95% CI 26.54 - 30.17; range: 16-38) (Figure 2).

The mean HAMD score after one month of treatment with an antidepressant was 18.93 (SE=1.44). The median HAMD score was 18.5 (95% CI 16.10 - 21.75; range: 8-37) (Figure 2).

There was a significant difference between the median HAMD score at baseline and post antidepressant therapy (Wilcoxon match-pairs test: W=119.00; p<0.001). There was an overall reduction of 35% in the HAMD scores after antidepressant therapy.




(Error bars represent 1st and 3rd interquartile)

**Figure 3.2. Median HAMD scores of the study population.**

## 3.4 Correlation between HAMD scores and cortisol levels of the study population at baseline and after treatment with an antidepressant.

There was a significant negative correlation between HAMD scores and cortisol levels at baseline (Spearman r=-0.38; p=0.048) (Figure 3).

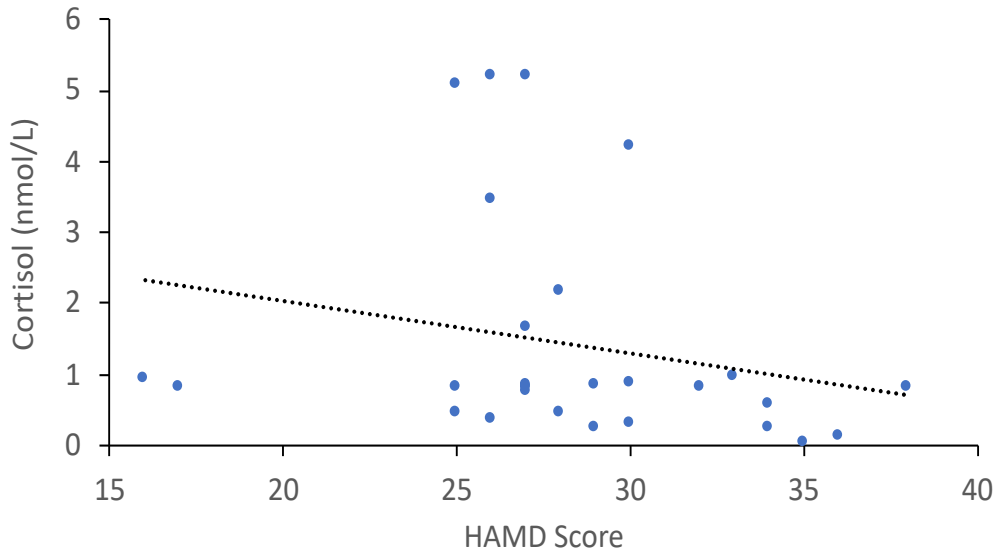



**Figure 3.3. Correlation between HAMD scores and cortisol levels at baseline.**

There was no correlation between the HAMD scores, and the cortisol levels post one-month treatment with an antidepressant (Spearman r=0.16; p=0.422) (Figure 4).

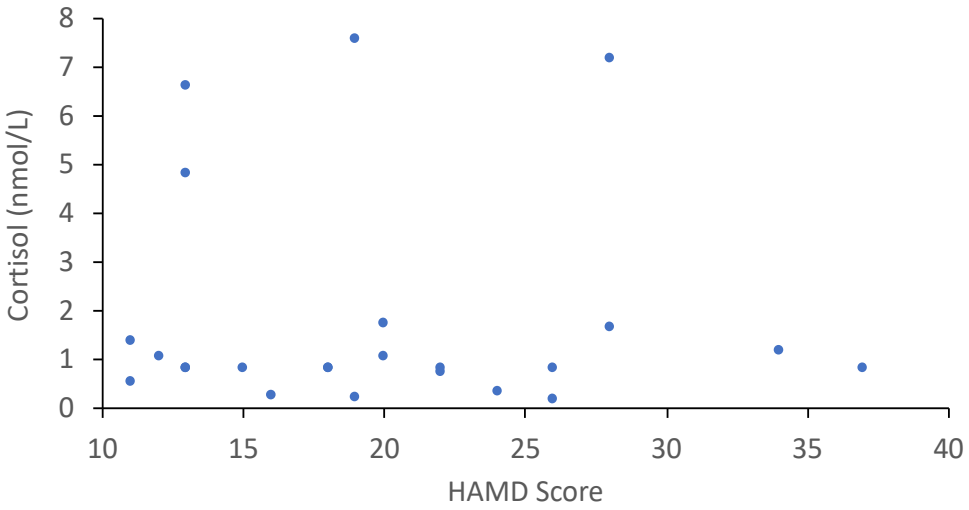



**Figure 3.4. Correlation between HAMD scores and cortisol levels post one-month treatment with an antidepressant**

## 3.5 Comparisons between median HAMD scores (at baseline and after one month of treatment) and socio-demographic characteristics of the study population.

There were no statistically significant associations between the median HAMD scores at baseline and age (H=1.41; p=0.494); gender (U=19; p=0.532), race (U =25.5; p=0.373), habitation (U=41.00; p=0.646), schooling level (U=42.00; p=0.673), current employment (U=90.50; p=0.888) and family history of mental illness (U=50.00; p=0.370) in the (Table 2).

**Table 3.2. Comparisons between baseline median HAMD scores and socio-demographic characteristics.**

| Variables | Median HAMD scores [*Values are median, (inter-quartiles) and n]* | Statistics |
|---|---|---|
| Age | | |
| *18-34* | 27 (25, 30); 17 | |
| *35-50* | 28.5 (27, 30); 6 | |
| *51-65* | 29 (27, 33); 5 | H=1.41; p=0.494 |
| Gender | | |
| *Female* | 27.5 (26, 30); 26 | U=19; p=0.532 |
| *Male* | 30.5 (27, 34); 2 | |
| Race | | |
| *Black African* | 27 (25, 36); 25 | |
| *Coloured* | 34 (26, 30); 3 | U =25.5; p=0.373 |
| Habitation | | |
| *Married* | 28 (27, 31.5); 4 | |
| *Single* | 27.5 (26, 31); 24 | U=41.00; p=0.646 |
| School | | |
| *Secondary* | 26 (26, 31); 24 | |
| *Tertiary* | 27 (21.5, 31.5); 4 | U=42.00; p=0.673 |
| Employment | | |
| *Employed* | 28 (27, 30); 11 | |
| *Unemployed* | 27 (26, 33); 17 | U=90.50; p=0.888 |
| Family history | | |
| *No* | 28.5 (27, 32); 22 | |
| *Yes* | 26.5 (26, 28); 6 | U=50.00; p=0.370 |

There were no statistically significant associations between the median HAMD scores after one month of treatment with an antidepressant and age (H=0.58; p=0.749); gender (U=17.00; p=0.422), habitation (U=43.00; p=0.743), schooling level (U=45.50; p=0.870), current employment (U=88.00; p=0.796), family history of mental illness (U=66.00; p=1.00). However, the median HAMD scores were significantly lower in Black compared to the Coloured participants after one month of treatment (U=8.00; p=0.028) (Table 3).

**Table 3.3: Comparisons between median HAMD scores after one month of treatment with an antidepressant and socio-demographic characteristics.**

| Variables | Median HAMD scores [*Values are median, (inter-quartiles) and n]* | Statistics |
|---|---|---|
| Age | | |
| *18-34* | 20 (13, 26); 17 | |
| *35-50* | 16 (13, 19); 6 | |
| *51-65* | 18 (15, 20); 5 | H = 0.58; p = 0.749 |
| Gender | | |
| *Female* | 18 (13, 22); 26 | |
| *Male* | 14.5 (9, 20); 2 | U = 17.00; p = 0.422 |
| Race | | |
| *Black African* | 18 (13, 22); 25 | |
| *Coloured* | 26 (26, 34); 3 | **U = 8.00; p = 0.028** |
| Habitation | | |
| *Married* | 19 (13.5, 21); 4 | |
| *Single* | 18.5 (13, 26); 24 | U = 43.00; p = 0.743 |
| School | | |
| *Secondary* | 18.5 (12.5, 25); 24 | |
| *Tertiary* | 18.5 (12.5, 25); 4 | U = 45.50; p = 0.870 |
| Employment | | |
| *Employed* | 19 (13, 26); 11 | |
| *Unemployed* | 18 (13, 22); 17 | U = 88.00; p = 0.796 |
| Family history | | |
| *No* | 19 (13, 24); 22 | |
| *Yes* | 14.5 (13, 28); 6 | U = 66.00; p = 1.00 |

## 3.6 Comparisons between median cortisol levels (at baseline and after one month of treatment) and socio-demographic characteristics.

There were no statistically significant associations between the median cortisol levels at baseline and age (H=2.55; p=0.280); gender (U=10.50; p=0.167), race (U=31.0; p=0.629), habitation (U=29.00; p=0.212), schooling level (U=47.50; p=0.974), and current employment (U=93.00; p=0.981) (Table 4). However, participants with a family history of mental illness had significantly higher cortisol levels compared to those who did not report a family history of mental illness (U=24.00; p=0.019) (Table 4).

**Table 3.4. Comparisons between baseline median cortisol levels and socio-demographic characteristics.**

| **Variables** | **Median cortisol levels** [*Values are median, (inter-quartiles) and n]* | **Statistics** |
|---|---|---|
| Age | | |
| *18-34* | 0.80 (0.47, 0.93); 17 | |
| *35-50* | 0.84 (0.29, 3.45); 6 | |
| *51-65* | 0.83 (0.83, 0.95); 5 | H =2.55; p=0.280 |
| Gender | | |
| *Female* | 0.80 (0.47, 1,65); 26 | |
| *Male* | 0.50 (0.25, 0.76); 2 | U=10.50; p=0.167 |
| Race | | |
| *Black African* | 0.80 (0.47, 0.95); 25 | |
| *Coloured* | 0.58 (0.14, 5.09); 3 | U=31.0; p=0.629 |
| Habitation | | |
| *Married* | 0.50 (0.25, 1.20); 4 | |
| *Single* | 0.80 (0.52, 1.55); 24 | U=29.00; p 0.212 |
| School | | |
| *Secondary* | 0.80 (0.47, 1.55); 24 | |
| *Tertiary* | 0.86 (0.47, 1.29); 4 | U=47.50; p=0.974 |
| Employment | | |
| *Employed* | 0.80 (0.47, 2.15); 11 | |
| *Unemployed* | 0.80 (0.58, 0.93); 17 | U=93.00; p=0.981 |
| Family history | | |
| *No* | 0.80 (0.38, 0.88); 22 | |
| *Yes* | 2.80 (0.80, 5.21); 6 | **U=24.00; p 0.019** |

There were no statistically significant associations between the median cortisol levels after one month of treatment with an antidepressant and age (H=0.36; p=0.837); gender (U=25.00; p=0.929), race (U=25.00; p=0.929), habitation (U=47.00; p= 0.948), schooling level (U=37.50; = 0.491), current employment (U=62.50; p=0.145) and family history of mental illness (U=65.00; p=0.955 ) (Table 5).

**Table 3.5. Comparisons between median cortisol levels after one-month treatment with an antidepressant. and socio-demographic characteristics**

| Variables | Median cortisol levels [*Values are median, (inter-quartiles) and n]* | Statistics |
|---|---|---|
| Age | | |
| *18-34* | 0.80 (0.38, 1.37); 17 | |
| *35-50* | 0.80 (0.80, 4.83); 6 | |
| *51-65* | 0.80 (0.80, 0.80); 5 | H = 0.36; p = 0.837 |
| Gender | | |
| *Female* | 0.80 (0.74, 1.18); 26 | |
| *Male* | 1.05 (0.38, 1.72); 2 | U = 25.00; p = 0.929 |
| Race | | |
| *Black African* | 0.8 (0.51, 1.37); 25 | |
| *Coloured* | 0.80 (0.51, 1.37); 3 | U = 33.00; p = 0.738 |
| Habitation | | |
| *Married* | 0.80 (0.59, 1.26); 4 | |
| *Single* | 0.80 (0.63, 1.28); 24 | U = 47.00; p = 0.948 |
| School | | |
| *Secondary* | 0.80 (0.45, 1.28); 24 | |
| *Tertiary* | 0.80 (0.80, 4.19); 4 | U = 37.50; p = 0.491 |
| Employment | | |
| *Employed* | 0.8 (0.20, 1.07); 11 | |
| *Unemployed* | 0.80 (0.80, 1.37); 17 | U = 62.50; p = 0.145 |
| Family history | | |
| *No* | 0.80 (0.74, 1.18); 22 | |
| *Yes* | 0.80 (0.51, 1.67); 6 | U = 65.00; p = 0.955 |

## 3.7 Comparisons between the change in median HAMD scores (from baseline to after one month of treatment) and socio-demographic characteristics.

There were no statistically significant difference between the change in median HAMD scores (from baseline to after one month of treatment) and age groups (H=3.36; p=0.186), gender (U=16.00; p=0.372), race (U=13.50; p=0.08), marital status (U=41.50; p=0.700), level of education (U=40.0; p=0.600), employment (U=89.50 p=0.851), and family history of mental illness (U=55.00; p=0.538) (Table 6).

**Table 3.6. Changes in median HAMD scores pre- and post- antidepressant treatment and socio-demographic characteristics.**

| Variables | Change in HAMD score (n=28) [*Values are median, (inter quartiles) and n]* | Statistics |
|---|---|---|
| Age group | | |
| *18-34 years* | -7.0 (-12.0, -3.0); 17 | |
| *35-50 years* | -12.0 (-14.0, -10.0); 6 | |
| *51-65 years* | -12.0 (-15.0, -9.0); 5 | H = 3.36; p = 0.186 |
| Gender | | |
| *Female* | -9.0 (-14.0, -3.0); 26 | |
| *Male* | -16.0 (-25.0, -7.0); 2 | U = 16.00; p = 0.372 |
| Race | | |
| *Black African* | -9.0 (-15.0, -6.0); 25 | |
| *Coloured* | 0.0 (-10.0, 1.0); 3 | U = 13.50; p = 0.08 |
| Habitation | | |
| *Married* | -8.0 (-17.0, -7.0); 4 | |
| *Single* | -9.5 (-14.5, -3.0); 24 | U = 41.50; p = 0.700 |
| School | | |
| *Secondary* | -10.0 (-15.0, -3.5); 24 | |
| *Tertiary* | -8.5 (-9.5, -5.5); 4 | U = 40.0; p = 0.600 |
| Employment | | |
| *Employed* | -10.0 (-14.0, -4.0); 11 | |
| *Unemployed* | -8.0 (-15.0, -3.0); 17 | U = 89.50 p = 0.851 |
| Family history | | |
| *No* | -9.0 (-15.0, -6.0); 22 | |
| *Yes* | -7.0 (-14.0, -1.0); 6 | U = 55.00; p = 0.538 |

It was noted that the change in HAMD scores was much lower in the 18-34 year age group (-7) compared to the 35-50 year (-12) and the 51-65 year age group (-12); in females (-9.0) compared to males (-16.0); in the coloured race (0.0) compared to the black African race (-9.0); in married participants (-8.0) compared to single participants (-9.5); in those with tertiary (-8.5) compared to secondary schooling level (-10.0); in those currently unemployed (-8.0) compared to those

employed (-10.0) and in those with a family history of mental illness (-7.0) compared to those without (-9.0) (Table 6).

### 3.8 Comparisons between the change in median cortisol levels (from baseline and after one month of treatment) and socio-demographic characteristics

It was noted that the change in cortisol levels was lower in the 18-34 year (-0.06) compared to 51-65 year age group (-0.03) and 35-50 year age group (-0.29); in females (-0.07) compared to males (-0.55); in the black African race (-0.06) compared to coloured race (0.61); in single participants (-0.07) compared to married participants (0.34); in those with secondary schooling level (-0.05) compared to tertiary schooling level (0.27); in those currently unemployed (0.13) compared to those employed (-16.0), and in those without a family history of mental illness (0.05) compared to those with a family history of mental illness (-0.51) (Table 7).

**Table 3.7. Changes in median cortisol levels pre- and post- antidepressant treatment and socio-demographic characteristics.**

| Variables | Change in Cortisol levels (n=28) [*Values are median, (inter quartiles) and n]* | Statistics |
|---|---|---|
| Age group | | |
| *18-34 years* | -0.06 (-0.53, 0.69); 17 | |
| *35-50 years* | -0.29 (-0.09, 1.38); 6 | |
| *51-65 years* | -0.03 (-0.15, 0.99); 5 | H = 0.95; p = 0.623 |
| Gender | | |
| *Female* | *-0.07 (-0.53, 0.60); 26* | |
| *Male* | *0.55 (0.13, 0.96); 2* | U = 15.00; p = 0.326 |
| Race | | |
| *Black African* | *-0.06 (-0.48, 0.55); 25* | |
| *Coloured* | 0.61 (-4.29, 0.66); 3 | U = 35.00; p = 0.853 |
| Habitation | | |
| *Married* | *0.34 (-0.36, 0.76); 4* | |
| *Single* | -0.07 (-0.63, -0.51); 24 | U = 41.00; p = 0.646 |
| School | | |
| *Secondary* | *-0.05 (-0.51, 0.58); 24* | |
| *Tertiary* | *0.27 (-0.49, 3.72); 4* | U = 41.00; p = 0.646 |
| Employment | | |
| *Employed* | *-0.16 (-0.84, 0.27); 11* | |
| *Unemployed* | 0.13 (-0.13, 0.96); 17 | U = 61.00; p = 0.126 |
| Family history | | |
| *No* | 0.05 (-0.15, 0.66); 22 | |
| *Yes* | -0.51 (-4.41, 0.00); 6 | U = 338.00; p = 0.117 |

## 3.9 Comparisons between the antidepressant prescribed and change in median HAMD scores and cortisol levels from baseline to post-treatment.

Of the 28 participants who completed the study, 24 participants (85.7%) were prescribed citalopram (dose 10mg daily {n=5} and dose 20mg daily {n=19}). Three (10.7%) participants were prescribed fluoxetine (dose 20mg daily) and one (3.57%) on another antidepressant (other than citalopram or fluoxetine).

There were no statistical associations between the changes in median HAMD scores (U=31.50; p=0.728) nor changes in median cortisol levels (U=29.00; p=0.589) and the type of antidepressant used (Table 8). There was a significant association between 10mg dosage of citalopram compared to the higher dosage of 20mg and the changes in median cortisol levels (U=20.0; p=0.050) but not for the change in median HAMD scores (U = 47.50; p = 1.000) (Table 8).

**Table 3.8. Comparisons between changes in median HAMD scores and median cortisol levels and the type of anti-depressants prescribed.**

| **Variables** | **Antidepressant therapy prescribed** [*Values are median, (inter-quartiles) and n]* | | **Statistics** |
|---|---|---|---|
| | **Citalopram** | **Fluoxetine** | |
| ∆ HAMD | -8.5 (-13.5, -3.5); 24 | -11 (-15.0, -3.0); 3 | U = 31.50; p = 0.728 |
| ∆ Cortisol | -0.06 (-0.59, 0.63); 24 | -0.06 (-0.09, 0.99); 3 | U = 29.00; p = 0.589 |
| | ***Citalopram 10 mg*** | ***Citalopram 20 mg*** | |
| ∆ HAMD | -10.0 (-14.0, -1.0); 5 | -0.08 (-13.0, -4.0); 19 | U = 47.50; p = 1.000 |
| ∆ Cortisol | -4.12 (-4.29, -0.53); 5 | 0.00 (-0.16, 0.96); 19 | **U = 20.0; p = 0.050** |

# 4.Discussion

## 4.1 Cortisol levels

*a) Prior to treatment with antidepressants*

The HPA axis and cortisol level dysregulation is reported to play a significant role in the pathophysiology of depressive disorders (Holsboer, 1996; Nemeroff, 2005). This study found lower than normal levels of cortisol (hypocortisolism) in depressed participants prior to antidepressant therapy. A similar finding of hypocortisolism in depressed patients has been noted in other studies. Yehuda (1995) reported hyposecretion of cortisol in individuals who lived under conditions of ongoing or chronic stress. Oldehinkel (2001) also noted lower cortisol levels in persons with a chronic history (>2 years) of stress and depression. Bremmer (2007), referred to the concept of HPA axis exhaustion and low cortisol secretion, which results from chronic stress-related to psychological trauma, prolonged periods of work stress, or chronic low-grade immune activation. Penninx (2007) and Bremmer (2007) described a 'U-shaped' association between plasma cortisol (patient's plasma cortisol varying from normal to hypo- or hypercortisolaemia) and major depression in later life. Hypocortisolaemia occurred more commonly in the elderly subgroup and the authors associated this with physical frailty which was related to chronic exhaustion of the HPA axis and insufficient cortisol response to stressors.

This study's results are contrary to many studies, which found evidence of hyperactivity of the HPA axis (as indicated by higher daytime cortisol levels) among persons with depression (Sachar, 1973; Carroll, 1976; Pfohl, 1985; Stokes, 1984; Gold, 1995; Bhagwagar, 2005). Otte (2004), reported baseline cortisol levels of 4.2 nmol/L in participants presenting with depression compared to 3.6 nmol/L in those with no depression. Khan (2019) also found increased cortisol levels of 2.2 nmol/L in patients with depression compared to

1.46 nmol/L in healthy subjects (p=0.031). Vreeburg (2009), reported much higher average cortisol levels of 21.51 nmol/L in participants with major depressive disorder. When these results were extrapolated by Manthey (2011), they showed an average cortisol level of 15.7 nmol/L. Similarly, Shabaan (2015) also showed significantly higher cortisol levels amongst patients with depression (14.2 nmol/L) compared to healthy controls (11.5 nmol/L) ($p<0.01$). It is evident that despite the wide variation in the actual level of cortisol, many studies report elevated cortisol levels in depression compared to normal healthy individuals.

Chronic stress was considered as a possible explanation to account for the hypocortisolaemia finding in this study. Although longstanding stress and duration of depressive illness were not explicitly measured in this study, the low median cortisol level amongst the participants is likely a consequence of a community suffering from a high burden of chronic stress and social stigma. Chronic and ongoing stress is a common occurrence in South African. Many individuals are exposed to the risk factors of depression, such as lower educational attainment, poverty, poor access to adequate health care, lack of stable marriage and unemployment (Hamad, 2007). These individuals have higher perceived stress and have limited support from their community, as there is still an ongoing stigma towards mental illness (Hamad, 2007). The majority of the participants in our study were either single, separated or divorced, resulting in an impoverished support base (Ranga, 2019) or unemployed, which is associated with financial instability (Ranga, 2019). As the participants in our study had a younger profile (not consistent with that of Penninx (2007)), it is assumed that the hypocortisolism finding in our study was a result of the exhaustion of the HPA axis from chronic stress rather than physical frailty. It is recommended that future research should include a measure of longstanding stress or duration of depressive illness into their methodology, especially in a South African context. Our study also found that females were at a higher risk for chronic stress and hypocortisolism, which was similar to the findings of Bremmer (2007). It is also recommended that health care workers should be made aware of this and increase screening measures and early detection of depression in this high-risk group.

It is also possible that the low levels of cortisol could be attributed to methodological factors in this study, such as the time that the cortisol samples were collected and the waking times of the participants. In a meta-analysis by Miller (2007), which looked at the correlation between chronic stress and HPA axis, it was found that cortisol levels are usually elevated when the cortisol samples are acquired in the early morning and lowest in the evening. Laudat (1988) found that cortisol levels were the highest in the early hours of the morning between 8 to 9 am and decreases through the day with the nadir at around 11 pm. Nieman (2017b) reported that salivary cortisol concentrations vary diurnally, with levels around 15.4 nmol/L at 8 to 9 am and about 2.8 nmol/L at 11 pm.

In this study, the cortisol samples were collected on the same day of participants' appointment with their treating psychiatrist. This method was adopted after consideration of the participants' social circumstances and to minimize travel costs. As a result of this method, cortisol samples were collected any time between 8 am to12 pm (average was approximately 11 am) after the participants had finished their consultation their psychiatrist (this included the waiting time for file collection, nurses review, psychiatrist appointment and possibly waiting time to collect medication). Thus, the cortisol levels at this time of day would be well past the peak levels of 8-9 am.

Further, Seth (2016) reported that highest cortisol concentrations are detected about 30-45 minutes after waking. Many of the studies cited were performed in inpatient settings, and the cortisol samples were collected soon after waking. This study, however, was conducted in an outpatient clinic. Kane (2006) reported that the majority of patients utilising public health care facilities in South Africa use public transportation as they do not own cars. Therefore, for patients to arrive timeously for their clinic appointments, their waking times would most likely be very early.

The combination of early waking times and the late collecting times of samples could be significant contributors to the findings of low cortisol levels. Further research, in a South African setting, should be done in an inpatient setting to get a

better understanding of the effect of the method of sampling on the results obtained.

*b) Post-treatment with antidepressants*

Our study found that the median cortisol levels after one month of treatment with an antidepressant remained unchanged. Similarly, Keating (2013) also reported relatively unchanged cortisol levels after SSRI therapy (88.3 ng/ml ±6.8 before and 84.3 ng/ml ± 6.2 after SSRI). Keating (2013) hypothesized that this may be attributed to only collecting a single cortisol sample (as was done in our study). They suggest that they might have shown a change in cortisol concentrations if there were multiple samplings done throughout the day, as individuals with depression often demonstrate an abnormal diurnal rhythm of cortisol.

In addition to the cortisol levels remaining unchanged following treatment, the levels in this study were also below normal. Hypocortisolism resulting from depression has been reported previously; however, in the majority of the literature, there is HPA axis hyperactivity which normalises with antidepressant therapy (Kapitany, 1999, Dziurkowska, 2013, Manthey, 2011). Antidepressants help in regulating the HPA axis by intensifying the feedback loop inhibition, thus decreasing the HPA axis hyperactivity in depressed patients (Pariante, 2008). Kapitany (1999), showed that cortisol levels decreased in depressed patients, after a 2-day citalopram stimulation procedure (from 9.4 mg dl to 8.8 mg dl). Likewise, Dziurkowska (2013) illustrated salivary cortisol suppression from 45.76±36.37 ng/mL to 18.30±39.99 ng/mL after SSRI therapy. This improvement had also been reproduced by Manthey (2011), who described a reduction in the average cortisol level post antidepressant therapy. Manthey (2011) further differentiated cortisol level improvements according to the antidepressant treatment prescribed. Those using SSRI antidepressant therapy had an average of 15.8nmol/L (26.5% reduction), and TCA antidepressant therapy had an average of 18.3nmol/L (14.9% reduction) after one month of treatment initiation. Laakmann (2004), also reported normalisation of a hyperactive HPA axis with mirtazapine administration. They found the average cortisol level was 85.78 ± 37.16 nmol/L per hour before mirtazapine administration in depressed patients. This cortisol level reduced after

the administration of mirtazapine to an average 53.95 ± 14.79 nmol/L per hour by day 21 (37.1% reduction in cortisol levels). They concluded that mirtazapine had acutely inhibit cortisol secretion and resulted in normalisation of cortisol levels. Nandam (2020) found normalisation of cortisol levels with chronic antidepressant use. They described findings of reduced urinary cortisol levels after fluoxetine treatment after four months. Holsboer (1996), also described the clinical effectiveness of antidepressant therapy by looking at longitudinal studies of dexamethasone suppression studies. They noted a normal response to dexamethasone suppression after antidepressant use. Aihara (2007) demonstrated HPA axis normalisation in a group of depressed participants post antidepressant therapy.

Furthermore, studies, where an initial hypocortisolism at baseline was found, have reported a later cortisol normalization after antidepressant therapy (Murck (2003). The type of depression in this subgroup was termed atypical depression as they had reversed vegetative signs. This group were prescribed monoamine oxidase inhibitors (MAOI) and hypericum extract, which lead to an activation of HPA axis activity and thus normalisation of the HPA axis. This absence of normalisation in our study could be attributed to the use of SSRI and not MAOI as used in Murck (2003). MAOIs are not routinely used in South Africa as a first-line agent due to potential adverse effects (The South African Society of Psychiatrists treatment guidelines for psychiatric disorders, SASOP, 2013).

However, Dziurkowska (2013) reported in their study, that after utilisation of SSRIs (such as sertraline, citalopram, escitalopram, and paroxetine) that there was an increase in cortisol levels around day 30 (but lower than the initial pre-treatment cortisol level) with a subsequent decrease thereafter. It is possible that the failure of this study to show HPA axis normalization after SSRI treatment could be due to the timing of the cortisol collection (cortisol sampling was done at day 28) as compared to later in the above study. Another factor was that Dziurkowska (2013) used participants from Poland, which is a more developed country and with different socioeconomic considerations compared to a South African population sample.

These studies show an improvement with antidepressants, differentiating them from this study. It is essential to consider that there were many methodological differences such as duration of the studies (an extended period showed a more considerable improvement in cortisol levels) and type of intervention used (different antidepressant therapies were prescribed). Similar further research should be conducted, perhaps with an extension of the duration of the study, as well as, to assess the difference in response to various antidepressant treatments in a South African context.

Additional factors accounting for low cortisol levels before the initiation of antidepressants include the late salivary cortisol collection times and early waking times of participants as discussed above.

*c) Associations between the change in cortisol levels pre and post-treatment and other variables*

This study found no significant change in the cortisol levels pre and post-treatment with an antidepressant. Nor any significant association with any variables and the overall change in median cortisol levels pre and post-treatment.

Many studies describe the association between change in cortisol levels pre and post antidepressant therapy. There is, however, a dearth of knowledge regarding the association between the change in cortisol levels and other variables pre and post antidepressant therapy. Majority of studies have collected sociodemographic data of their participants but have not further extrapolated its association with cortisol levels. In our context, the sociodemographic data may indicate the presence of chronic stress; hence other studies demonstrating that association would be beneficial to this study.

Keating (2013) reported no change in cortisol levels pre and post antidepressant (SSRI) therapy and found no difference in response between males and females after comparing the cortisol levels. Similarly, this study showed no association between gender and change in cortisol levels.

## 4.2 HAMD scores

*a) Prior to treatment with antidepressants*

This study found a median HAMD score at entry to be 27.5, which is above the cut-off score for severe depression (≥23). Similarly, in other studies, high HAMD scores in depressed patients prior to treatment have been reported. Zhou (2017), in a study of 596 participants (299 were diagnosed with MDD, and 297 were the control group) showed an average HAMD score of 23.8 before treatment. Young (2004), found a response to fluoxetine in 20 treatment naïve participants with MDD, who reported an average HAMD score of 24 ± 3 at entry to study. Yehuda (1996) compared cortisol regulation in PTSD and MDD participants and reported a HAMD score of 29.46 ± 7.3 in the male, treatment naïve cohort group diagnosed with MDD. Gou (2015) studied 120 female patients diagnosed with MDD and reported an average HAMD scores of 22.4 before any antidepressant intervention.

This study found higher average HAMD scores compared to the majority of the above studies, which could suggest a late presentation to health care establishments. Thus, strategies targeting high-risk patients in primary care centres should be a crucial factor in protocol development.

*b) Post-treatment with antidepressants*

It must be noted that an improvement in HAMD score does not always equate to adequate response/resolution of MDD. Only those with a >50% improvement in HAMD scores are labelled as responders to antidepressant therapy. This study was able to show a statistically significant improvement in the median HAMD score (35%) after treatment with SSRI antidepressant therapy. While this study showed an improvement in average HAMD scores, it was not enough to mark the entire group as responders after one month of antidepressant treatment. Many of the participants still described symptoms of their depression which would place them in the category of moderate depression.

Nikkheslat (2019) also reported a variable reduction in HAMD scores in their sample group after six weeks of antidepressant therapy. They conducted a cross-sectional study dividing participants into three categories depending on the response to antidepressant therapy (responders, non-responders and those not yet on treatment). The average HAMD scores were as follows: untreated depression (20.3), non-responders (18.1) and responders (3.6). Approximately two times more participants were non-responders compared to responders. Similarly, this study found more non-responders compared to responders after antidepressant therapy.

One reason for the suboptimal response to antidepressants could be due to the short duration of this study. However, this inadequate response to antidepressants goes against the findings found by Szegedi (2009), who demonstrated that early improvement (less than two weeks) with antidepressant therapy using the HAMD, was a good predictor of stable response and remission. Another reason for partial response to treatment may be because no other form of intervention such as psychotherapy or social worker support was offered as an adjunct to pharmacotherapy. The participants' self-perceived lack of change/ improvement in their social or financial problems could have impacted on their response to pharmacotherapy. Both low support (Patten, 2010) and inadequate economic resources (Bracke, 2013) are risk factors for depression. A South African community with limited socioeconomic resources likely requires added support in the form of support groups, individual therapy and social worker intervention in addition to pharmacotherapy to manage their depression adequately. Another reason for the suboptimal response to antidepressants was the possible misdiagnosis of the participants. There could have been a missed personality disorder or bipolar depression. Their coping skills were also not assessed. All of these could have attributed to the suboptimal response noted in this study.

This study is in contrast to other studies where participants showed an adequate response to treatment (HAMD improvement by at least 50%). Studies such as Young (2004), indicate an improvement in HAMD scores from a baseline of 24±3 (66.67% reduction) to 8±2.7, in the treatment responder group, after six weeks of fluoxetine. Similarly, Tadić (2010) showed improvement in depression

(improvement of HAMD scores) after other subtypes of antidepressant therapy were commenced. Tadić (2010) assessed the response of 84 depressed participants, with baseline HAMD scores of 16.9±4.5, after sertraline administration. After one month of sertraline antidepressant therapy, 60% of the participants showed a greater than 20% decrease in HAMD score and 15% had greater than 50% decrease in HAMD score (actual HAMD scores post antidepressant therapy were not provided).

*c) Associations between change in HAMD scores and other variables*

There was no significant association between the overall change in HAMD scores pre and post-treatment with most demographic and clinical variables. It was noted that median HAMD scores were lower in Black African participants compared to the Coloured participants after one month of antidepressant therapy. The only variable known to have changed in this sample group was the addition of antidepressant treatment. Therefore, it could be extrapolated that the difference in response rate amongst the two racial groups would be their sensitivity to SSRI antidepressant therapy.

Others have investigated remission rates of depression amongst different racial groups. Lesser (2007) found no statistical significance amongst different racial groups and HAMD scores but did find lower remission rates amongst Black American participants compared to White participants and Hispanic participants. This finding was also noted by Fortuna (2010), who showed poor treatment retention and thus reduced remission rates of depression amongst the ethnic and racial minority in the United States of America. Poor treatment retention in African American patients was attributed to superficial disclosing of information to health care workers, abrupt termination of counselling, their belief that the mental illness would improve by itself and the lack of racial concordance between patient and health care provider. Further research in a South African setting would need to be done to assess poor treatment retention. A similar discordance between health care provider and participant likely occurs in a South African context, which may attribute to the participants having a reduced remission rate.

This study also showed no statistically significant associations between the changes in median HAMD scores and the type of antidepressant used. Both treatment options were in the same antidepressant class (SSRI). Therefore, this could be a reason that there was no difference in efficacy between citalopram and fluoxetine.

In the South African public sector, there are limited available treatment options, with SSRIs being the first line of treatment (The South African Society of Psychiatrists treatment guidelines for psychiatric disorders, 2013). The increased use of SSRIs would only be a quandary if the efficacy of SSRI were suboptimal as compared to other antidepressant agents. Geddes (2007) analysed 98 randomised control studies to assess the effectiveness of SSRI treatment as well as compare SSRI treatment with the older tricyclic antidepressants (TCA). Geddes (2007) found no clinically significant differences in efficacy between SSRIs and TCA; instead, treatment choice should be based on patient preference, drug toxicity and cost. Similarly, Hirschfeld (1999) reviewed three meta-analysis and 20 clinical trials and found that SSRIs had comparable efficacy to TCA for the treatment of severe or melancholic depression. Patris (1996) performed a double-blind, randomized trial comparing citalopram and fluoxetine in patients with unipolar depression. They found a reduction in the HAMD score in both groups with no statistically significant differences between treatments, including adverse effects. These studies show similar efficacy between SSRIs and TCA; thus, the treatment options used are justified.

There was no significant association between the 10mg dosage of citalopram compared to the higher dosage of 20mg and the changes in median HAMD scores. No significant association between the dosages could be attributed to impaired tolerance of medication at a higher dosage; and the antidepressant effect of citalopram is still present with the 10mg dose (Bech, 2002). Torta (2012) describes that a patient's motivation and adherence is linked to the tolerability of treatment. Therefore, it is essential to look at both the side effect profile of antidepressant therapy at higher doses as well as the clinical response with lower doses. Torta (2012) analysed studies from colleagues, many from Italy (a first

world country); a nation of increased resources (possibly psychotherapy) compared to South Africa. Increased resources afford health care workers to adjunct antidepressant (e.g. with psychotherapy) in their participants. Therefore, health care workers would have the advantage of slow up-titration of the antidepressants. In a South African setting, where there are limited resources, a health care worker may not have the luxury of slow up-titration.

## 4.3 Correlations between HAMD scores and cortisol levels

This study found a significant inverse relationship between cortisol and HAMD scores at baseline; as the HAMD scores increased, the cortisol levels decreased. This inverse relationship is in keeping with multiple studies that suggest that chronic stress leads to hypocortisolaemia. Such as described by Oldehinkel (2001), who noted that low cortisol levels were present in those with chronic depression (>2 years). Penninx (2007) also described hypocortisolaemia in the elderly subgroup, which they attributed to physical frailty. The inverse relationship between cortisol and HAMD scores were also noted by Bremmer (2007), who explored HPA axis exhaustion from chronic stress resulting in hypocortisolaemia.

This study's findings are, however, are in contrast with some studies where a positive correlation was noted. Studies have shown an association between HAMD scores and cortisol by illustrating hypercortisolaemia with MDD (high HAMD scores). Maes (1986) demonstrated elevated cortisol levels in 100 depressed patients post dexamethasone suppression test at baseline, thus showing a positive correlation between cortisol levels and HAMD scores. They further stratified their participants into major and mild depression based on the severity of elevation of their cortisol level. Participants with major depression had both higher HAMD scores as well as cortisol values compared to those who had minor depression. The highest cortisol levels, at the various measurement times (8 am, 4 pm and 11 pm), in the participants were the best predictors for severity of the depression when measured with the HAMD score. Yoon (2015) showed that there were significant positive correlations between plasma cortisol levels and the

depression component of the HAMD and vice versa; where cortisol levels rose with the severity of depression (as shown by increasing HAMD scores).

There was no correlation between the HAMD scores and the cortisol levels one-month post-treatment with an antidepressant. This lack of an association could be explained on the basis that our patients already had hypocortisolaemia at baseline. On the other hand, other studies which have shown a normalisation of the HPA axis after antidepressant therapy. Studies such as Laakmann (2004), showed elevated cortisol levels in 12 depressed patients, which improved with resolution of depression. Similarly, Kapitany (1999), showed a reduction in cortisol levels (from 9.4 mg dl to 8.8 mg dl) in 16 patients diagnosed with acute major depression after citalopram 20 mg, 2-day stimulation procedure; the participants in this trial had HAMD scores ranging from 19-32, but due to the short trial, HAMD scores were not repeated. Likewise, Navines (2007), studied a group of participants with MDD (HAMD score >17, mean HAMD score 22.2) and demonstrated a decrease in HPA axis overactivity after administration of citalopram for eight weeks; these patient s showed a reduction in ACTH, cortisol and HAMD score (mean 11.48).

## 5. Limitations

The following limitations of this study are noted:

1) For meaningful statistical analysis, the sample size was determined to be 51 patients. However, only 28 patients completed the study because a significant number failed to keep their one month follow up appointment and had to be labelled as dropouts.
2) Recruitment was difficult as large numbers of patients referred for participation in the study had to be excluded. Some were not antidepressant therapy naïve at baseline (treatment already initiated by primary health care professionals), and others had co-morbid medical condition or another axis I psychiatric diagnosis (which is common in this population).
3) Salivary cortisol samples should have been collected at peak cortisol secretion based on circadian rhythm, which is 30-40 minutes after waking. Because of the outpatient nature of this study and the time taken for patients to have their samples collected, it ended up being many hours after waking and may have impacted on the cortisol levels recorded.
4) To assess the HPA axis response to antidepressants, patients needed to be adherent to their medication. There is no objective test that can reliably ascertain adherence to antidepressants at our clinics; hence the researchers relied on a pill count at the second visit. This method is unreliable and subject to manipulation by patients to appease the doctor.
5) The validity of the HAMD in this sample population may be affected by the fact that this was a predominantly African population subgroup, with English not being their first language. As such, there may have been a misunderstanding of some of the English nuances in the HAMD, resulting in misinterpretation and thus affecting the grading scales.

## 6. Recommendations

1) It is recommended that further studies be done in this area with larger sample size, including variables such as participants' waking times, grading stress levels and childhood trauma, objective monitoring of treatment adherence, and longer duration to allow more time for potential cortisol normalization post antidepressant therapy. It would also be advantageous that future studies utilise a validated rating scales in the participant's home language.

2) While this study showed an improvement in average HAMD scores, it was not enough to mark the entire group as responders after one month of antidepressant treatment. Many of the participants still described symptoms of their depression which would place them in the category of moderate depression. In addition, this study found that the participants had low cortisol levels at entry to study, suggesting that they may be experiencing chronic stress which was contributing to their depression. It is therefore recommended that mental health service providers ensure that all depressed patients be provided with a more holistic management, including psychotherapy and social worker intervention to facilitate a change in the patient's home life.

# 7. Conclusion

There is limited research on cortisol activity amongst depressed South Africans as well as the response with an antidepressant on normalising cortisol levels and improving depressive symptoms. This study illustrated a significant improvement in the severity of depression in the participants after one-month of antidepressant treatment. This was evidenced by improved HAMD scores. The authors are of the opinion that the patients would have yielded much better responses had the treatment been for a longer duration and included adjunctive therapy such as psychotherapy and social worker intervention.

However, this study did not find high circulating cortisol due to HPA axis dysregulation amongst the depressed patients, but rather hypocortisolaemia. It is postulated that, similar to other studies which reported these findings, the low cortisol levels may be attributed to chronic stress experienced by the participants as a result of their lower socioeconomic and disadvantaged backgrounds. Treatment with an antidepressant had no impact on the cortisol levels. Hence, cortisol levels in this population is not always useful as a predictor of the severity of depression or serves as a measure of the response to treatment.

It is recommended that further studies be conducted in a larger sample group and for a longer duration to investigate this population’s baseline cortisol level and response to antidepressant therapy. The study should also look at the effect of severity and duration of social stressors on cortisol levels.

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