

1 Rise and Fall; Cortisol levels Pre and
2 Post Antidepressant therapy in
3 Depression
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15 A research report submitted to the Faculty of Health Sciences, University of the
16 Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the
17 degree of Master of Medicine in Psychiatry
18

19 Johannesburg, 2020
20

21 **Declaration**

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

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31 Dr Priscilla Iswari Vythilingum Naidu

32

33 ...24...day of11....2020.....in...Johannesburg.....

34

35 **Dedication**

36

37 To my family

38 Without whom none of my success would be possible

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57

58 **Abstract**

59 A consistent finding in psychiatry is Hypothalamic-Pituitary-Adrenal (HPA) axis
60 activation in major depression. These findings would usually be expressed as
61 hypercortisolism at baseline (Alheira, 2005; Mackin, 2004; Manthey, 2011; Young,
62 2004). However, there are contradicting studies illustrating hypocortisolism
63 secondary to chronic stress or depression as a result of fatiguing of the HPA axis
64 (Yehuda, 1995; Oldehinkel, 2001).

65

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

70

71 **AIM:**

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

75

76

77 **METHOD:**

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

82 **RESULTS:**

83 Forty-three participants were initially recruited for this study, of which only 28 had
84 complete data sets at the end of the study. The majority of the sample population
85 consisted of black (89.3%), unmarried (85.7%) and unemployed (61.7%) females
86 (92.9%), primarily in the 18-34 year old age group (60.7%) who had attained a
87 secondary school level of education (85.7%).

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

102 CONCLUSION:

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

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120

121 **Acknowledgements**

122

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124 Academic Hospital and National Department of Health for permission to undertake
125 this research in their establishment and to the patients who participated in the
126 study.

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234

235

236

237 1. Introduction

238 1.1. Background

239 *Epidemiology of depression*

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

254

255 The World Health Organisation (WHO, 2017) found an increase in the prevalence
256 of depression by 18% between 2005 and 2015, with an estimate of 322 million
257 people globally living with depression in 2017. This has also been noted in the
258 United States, where there was an increase in prevalence of major depression,
259 from 3.33% to 7.06%, between 1991-1992 and 2001-2002 (Compton, 2006). In
260 sub-Saharan Africa, neuropsychiatric disorders account for roughly 10% of the
261 total burden of disease (Tomlinson, 2009). Focusing in on South Africa, Herman
262 (2009) analysed data collected from 4351 adults from different South African
263 regions and racial groups and found that the lifetime prevalence for any mental
264 disorder is 30.3%, with one of the commonest disorders being major depressive
265 disorder. Furthermore, this data was analysed by Tomlinson (2009) illustrating the
266 lifetime prevalence of a major depressive disorder was 9.7% (4.9% for the past 12
267 months) (Tomlinson, 2009). South Africa has a higher prevalence compared to

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

273

274 A systematic analysis for the global burden of disease study found major
275 depressive disorder to be amongst the top ten causes of YLD in every country in
276 2013 (Vos, 2015). According to Bateman's article in Noseweek (2014), one-
277 quarter of South Africans suffer from depression, leading to a 2.2% loss in annual
278 Gross Domestic Product (GDP) (R40.6 billion) and an estimated yearly loss of
279 earning per adult of 54 thousand Rands. Discovery Health (South Africa's most
280 substantial medical aid) revealed a 41% increase in mental health pay-outs (from
281 R96.7 million to R494.6 million), primarily to treat depression (Bateman, 2014). It is
282 essential to bear in mind that 80% of the South African population is managed
283 within the public sector and that financial statistics regarding depression,
284 specifically, are not available (Bateman, 2014). Bateman (2014) also reports that
285 230 suicides are attempted daily due to depression. Depression is a significant
286 burden on South Africa's GDP, health expenditure and social well-being. Improved
287 and focused prevention and treatment options for those at risk of depression or
288 diagnosed with depression is therefore vital.

289

290 *Risk factors for depression*

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

297

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

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

312

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

317

318 Depression also results from an interplay between genetics and the environment
319 (Ranga, 2019). As altered gene expression contributes a vulnerability towards the
320 development of depression as demonstrated in twin studies (Ranga, 2019). There
321 is also a higher heritability component in the development of female depression
322 compared to male depression (Seedat, 2009). Females were 1.75 times more
323 likely to experience lifetime depression and 2.17 times more likely to experience
324 12-month depressive episode compared to males (Tomlinson, 2009).

325

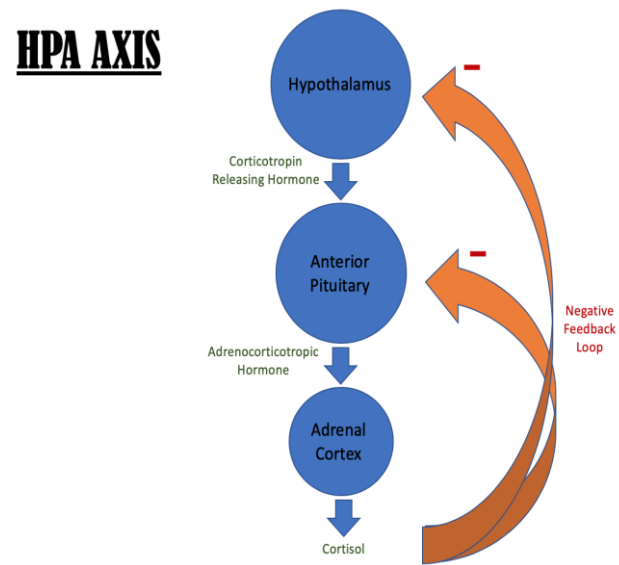
326 **1.2 Cortisol dysregulation in depression**

327 *Physiology of Cortisol*

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

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

342



343

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

345 The HPA axis is essential in regulating many peripheral functions such as the
346 metabolic system (regulates glucose storage and utilisation) and immune system
347 (involved in the inflammatory response and lymphocyte maturation) (Miller, 2007;
348 Mackin, 2004). The HPA axis is of particular interest in the area of psychiatry as
349 not only does it regulate the metabolic and immune system but is also able to
350 modulate the central nervous system directly. Regulation of the HPA axis appears
351 to be important in the pathophysiology of many psychiatric disorders. For example,
352 glucocorticoids regulate neuronal survival, neurogenesis, the sizes of complex
353 anatomical structures, such as the hippocampus which is essential for the
354 acquisition of new memories and the emotional appraisal of events (Pariante,
355 2008).

356

357 *Cortisol and Depression*

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

366

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

380

381 There is a large body of evidence that supports HPA axis dysregulation with major
382 depression. Pariante (2008) and Alheira (2005) showed non-suppression of the
383 HPA axis after an oral dose of synthetic glucocorticoid dexamethasone; where the
384 typical response should have been a potent feedback inhibition of the HPA axis.
385 Otte (2004) illustrated HPA hyperactivity in a depressed adult group of 693
386 participants in the United States patients by collecting a 24-hour urine sample and
387 testing for cortisol— patients with the highest levels of cortisol ($\geq 47.4\mu\text{g}/\text{day}$) had
388 two-fold increased odds for having depression. Otte (2004) also found that
389 increased cortisol levels were proportional to the severity of the depression
390 (patients with severe depression had $42\pm 28\mu\text{g}/\text{day}$ versus patients with no

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

401

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

412

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

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

429

430 Other symptoms described in the DSM 5 include changes in sleep pattern (DSM,
431 2013). Inadequate or poor sleep could be as a result of elevated cortisol, as
432 cortisol causes an increase in sympathetic nervous system activity and decreased
433 parasympathetic nervous system activity (McEwen, 2007). Resultant sleep
434 impairment compounds the cognitive impairment that results from depression.

435

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

456

457 *South African studies*

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

469

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

482

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

489

490 **1.3. Measuring cortisol levels**

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

503

504 For example, using blood substrate for cortisol measurement requires
505 venepuncture. This procedure is costly, invasive, requires medical staff,
506 specialised equipment and may require special handling as it may be considered a
507 biohazard. Another drawback is the assumption that further venepuncture at later
508 stages may elicit a cortisol response and thus give falsely elevated levels (Seth,
509 2016). In a clinical setting, a random total plasma cortisol sample (blood sampling)
510 would be useful in determining any abnormal variation of the diurnal secretion of
511 cortisol. If found, then further testing may be needed (Levine, 2007). However,
512 total cortisol may not be diagnostically useful in determining adrenal function. In
513 these cases, free cortisol may be a better test (hypoproteinaemia as binding
514 globulins need to be taken into account) (Levine, 2007).

515

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

521

522 Measuring salivary cortisol has many advantages such as being stress-free, non-
523 invasive, rapid, it allows for frequent sampling to be done, and there is no need for
524 trained staff or specialised equipment (Levine, 2007; Haeckel, 1993). It is also
525 convenient as sampling is not limited to a medical/laboratory setting (Levine,
526 2007). However, there are still some disadvantages such as poor compliance if
527 given to do in a home setting, insufficient salivary samples, samples may be taken
528 post eating, or drinking (affecting the cortisol concentrations), or samples may be
529 done when a patient has an oral lesion (blood affects the cortisol concentration).
530 Majority of these limitations can be resolved by collecting the samples in a clinical
531 setting (Levine, 2007) as it is operator dependent (Miller, 2007; Blair, 2017). Also,
532 to note, cortisol found in saliva would usually be lower than blood serum due to the
533 conversion of cortisol to cortisone (an inactive metabolite) in saliva (Seth, 2016).
534 Salivary cortisol sample reflects HPA activity for the past 10-60 minutes (Miller,
535 2007).

536

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

547

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

555

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

561

562 **1.3.1 Salivary collection, time and assay**

563 Providing a salivary sample for cortisol measurement is simple in both children
564 and adults, where they can either salivate directly into a tube or chew on a piece of
565 cotton wool (Blair, 2017). Studies have shown that the salivary concentration was
566 independent of flow rate and that there was no difference between whole salivary
567 cortisol concentrations compared to parotid salivary cortisol concentrations (Read,
568 1990).

569

570 Storage of these salivary samples is relatively uncomplicated and gives the
571 clinician time for analysis as samples can be kept at room temperature for three
572 days, up to seven days if kept at 4°C, and for later measurement, it can be stored
573 at -20°C for up to nine months (Read, 1990). A South African NHLS Memo dated
574 16 August 2016, describes the procedure of salivary cortisol sample collection and
575 storage. This memo states that the collection tube containing the saliva saturated
576 cotton wool should be sent to the laboratory after collection as it is stable at room
577 temperature 20-25°C for 24 hours. If transport is delayed then the sample can be
578 refrigerated as it is stable at 2-8°C for four days; however, if there is a longer delay
579 to processing, then the sample can be stored at -20°C for twelve months.

580

581 Salivary cortisol concentrations have diurnal variation, reaching a peak around the
582 time of awakening with a concentration of about 5.6 ng/mL (15.4 nmol/L) at 8 to 9
583 AM and a nadir after the onset of sleep about 1 ng/mL (2.8 nmol/L) at around 11
584 PM (Laudat, 1988; Levine, 2007). Therefore, collection times should be based on
585 this diurnal variation (Laudat, 1988). This recommendation is corroborated by Seth
586 (2016). Seth (2016) stated that cortisol concentrations are significantly increased
587 30-45 minutes post waking and a subsequent decline throughout the day and

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

591

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

603

604 **1.3.2 Factors affecting cortisol levels**

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

608

609 *Age*

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

617

618 *Gender*

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

623

624 *Medication*

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

637

638 *Medical conditions*

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

648

649 *Illicit substance use*

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

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

658

659 *Other major psychiatric illnesses*

660 Cortisol levels are particularly affected in women diagnosed with an anxiety
661 disorder where the cortisol was found to be blunted. However, in males, cortisol
662 was found to be elevated in those diagnosed with a social anxiety disorder (Zorn,
663 2017). For those diagnosed with schizophrenia, cortisol stress reactivity was
664 blunted in both men and women (Zorn, 2017). Patients who have developed post-
665 traumatic stress disorder also have altered cortisol levels; with hypocortisolism
666 frequently being found in previously healthy individuals who are now suffering from
667 ongoing stress (Heim, 2000; Smyth, 1997).

668

669 *Shift workers*

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

673

674 **1.4 Cortisol and antidepressants**

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

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

689

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

702

703 **1.5. Rating scale in depression**

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

711

712 Another benefit for the use of rating scales is to identify non-responders and those
713 with residual symptoms, as a lack of improvement/residual symptoms require a
714 change in treatment to avoid relapse (Szegedi, 2009; Pintor, 2003; Faravelli,
715 1986). Rating scales can also illustrate improvement, which is useful in patients

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

718

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

726

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

739

740 An advantage besides the continuous and widespread use of the HAMD is the
741 improved inter-rater reliability with the use of a structured interview guide (Cusin,
742 2009). The internal, inter-rater and retest reliability estimates are mostly suitable
743 for the global score but may be weaker for individual items (Bagby, 2004). The
744 HAMD is easy to score, and the cut-offs in scoring stratify the level of severity of
745 depression (Cusin, 2009). The HAMD is easily accessible as it is widely available
746 in the public domain and is not protected by copyright.

747

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

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

752

753 **1.6. Correlation between HAMD and salivary cortisol level**

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

765

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

773

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

777

778 **1.7 Purpose of the study**

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

785

786 **1.8 Hypothesis**

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

790

791 **1.9 Aim**

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

795

796 **1.10 Objectives**

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

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

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

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824 **2. Method**

825 **2.1 Study design:**

826 This study was a prospective and quantitative study.

827

828 **2.2 Site of study:**

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

834 for

835 **2.3 Participants:**

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

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

841

842 The exclusion criteria for this study were:

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

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

858

859 **2.4 Sample size**

860 The primary outcome measure was the difference in salivary cortisol values from
861 before treatment and four weeks post-treatment with an antidepressant using a
862 parametric t-test. A Power calculation was run using G Power (Faul, 2007).

863 Although the original power calculation was based on research with a group of
864 twelve patients by Laakmann (2004), using a mean difference of 7nmol/l and a
865 standard deviation of 3.5nmol/l, a probability p-value = 0.05 and an estimated
866 power of 0.85 a total sample size of 20 was found to be required to determine
867 statistical significance. A sample size of 20 participants was felt to be an
868 insufficient sample size. Therefore, the sample size was adjusted by changing the
869 effect size from 0.5 to 0.3 and using Cohen's D resulting in the new sample size
870 requiring 51 patients.

871

872 **2.5 Study Method:**

873 Participants were recruited during the period January 2017 to December 2017.

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

880

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

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

893

894 *Pre-treatment data collection:*

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

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

905

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

913

914 *Collection of salivary samples:*

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

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

922

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

929

930 The date, time and the allocated participant study number was documented on the
931 tube. The tube was taken to the laboratory within 24 hours. If there was any delay
932 in getting samples to the laboratory; then the samples were refrigerated between
933 2-8 degrees Celsius and later taken to the lab in a cooler box with ice.

934

935 *Cortisol assay:*

936 The salivary collection tubes were centrifuged at 3500g for 15 minutes, the cotton
937 wool plugs were subsequently discarded after the centrifuge, and the remaining
938 saliva samples were frozen at -20 °C to precipitate mucins for later analysis.

939 When the salivary assay was to be performed, the sample was thawed and
940 centrifuged at 1500 g for 15 min. All samples were assayed together in duplicate
941 using a commercial high-sensitivity radioimmunoassay for salivary cortisol EIA
942 (Salimetrics LLC). The samples were tested according to the manufacturer's
943 instructions. The lower limit of detection for the assay was 0.19 nmol/l. The intra-
944 and inter-assay coefficients of variation (CVs) were listed as < 6%. The cortisol
945 assays were tested in the physiology department at Witwatersrand medical school,
946 Johannesburg with the assistance of the University of the Witwatersrand's
947 Physiology team.

948

949 *Post-treatment data collection:*

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

953

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

960

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

966

967 **2.6 Ethical considerations**

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

971

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

977

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983 **2.7 Data Analysis**

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

993

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

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1014 **3. Results**

1015 Forty-three participants were initially recruited for this study, of which only 29
1016 attended their one-month follow-up. Of these 29 participants, one had an
1017 insufficient cortisol sample prior to antidepressant therapy. The final study
1018 population thus comprised of only 28 participants, who had complete data sets.
1019

1020 **3.1 Socio-demographic characteristics of the study population**

1021

1022 Analysis of the socio-demographic data showed that there was no statistically
1023 significant difference between the age groups ($\chi^2_3=9.85$; $p=0.009$). Nor was there
1024 a significant difference in the number of participants who were employed versus
1025 unemployed ($n=11$ vs 17) ($\chi^2_1=1.29$; $p=0.257$) (Table 1).

1026

1027 Statistically significant results found in the socio-demographic data were attributed
1028 to gender, race, relationship status, education level and family history of mental
1029 illness. The gender of the participants was significantly skewed towards females
1030 ($n=26$ vs 2) ($\chi^2_1=20.57$; $p<0.001$) (Table 1). Only two race groups (Black African
1031 and Coloured) were recorded amongst the participants, of which significantly more
1032 were Black African ($n=25$ vs 3) ($\chi^2_1=17.23$; $p<0.001$) (Table 1). Significantly more
1033 participants were unmarried ($n=24$) or were separated compared to participants
1034 who were married or living with a partner ($n=4$) ($\chi^2_1=14.29$; $p<0.001$). The level of
1035 education of the recruited participants showed that significantly more participants
1036 had a secondary school education ($n=24$) compared to primary ($n=0$) and tertiary
1037 ($n=4$) education ($\chi^2_2=35.43$; $p<0.001$). Significantly more participants ($n=22$)
1038 reported no incidence of mental illness in the family compared with those who had
1039 ($n=6$) ($\chi^2_1=9.14$; $p=0.002$) (Table 1).

1040

1041

1042 **Table 3.1: Frequency distribution of the demographic characteristics of the**
 1043 **study population.**

1044

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

1045

1046

1047 **3.2 Cortisol levels of the study population.**

1048 Cortisol samples were collected on all 43 participants at the initial visit, prior to
1049 antidepressant therapy consumption. However, cortisol concentrations were
1050 available for only 41 participants as 2 samples were insufficient for analysis. The
1051 cortisol levels of the 41 participants at entry into study ranged from 0.032 to 6.973
1052 nmol/L. The mean score was 1.63 nmol/L; the median was 0.83 nmol/L.

1053

1054 For the participants who had cortisol levels, both a pre and post antidepressant
1055 therapy (n=28), the mean cortisol level at baseline was 1.42 nmol/L (SE=0.31).

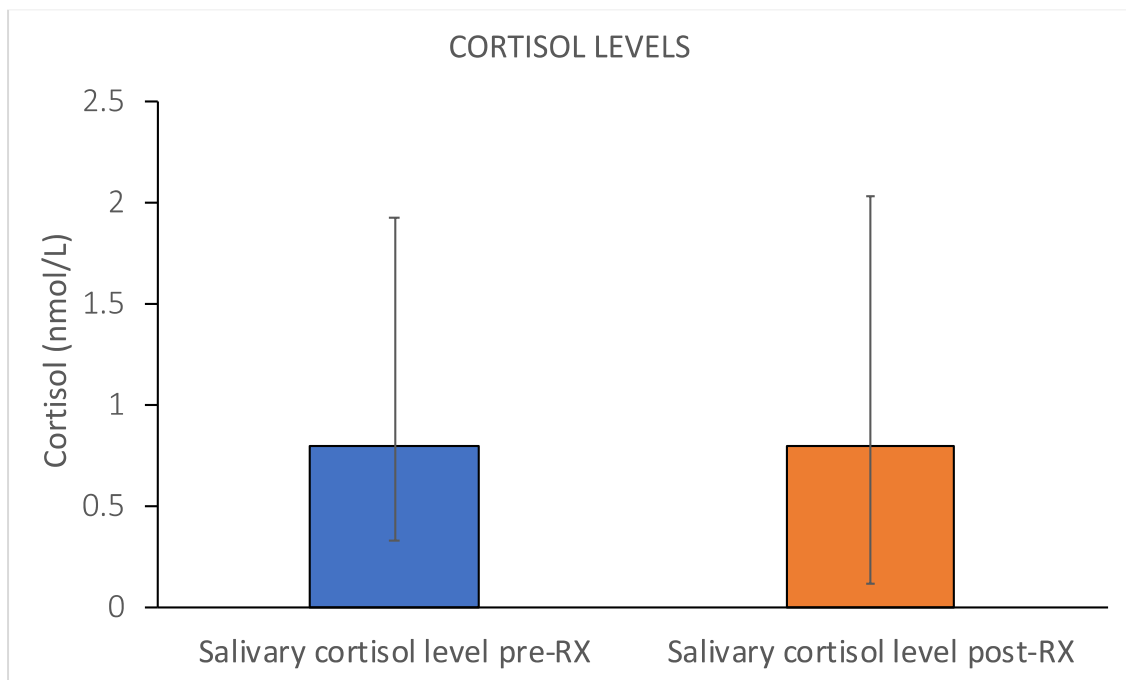
1056 The median was 0.80 nmol/L (95% confidence interval (CI) 0.82 - 2.02; range:
1057 0.032-5.207). The mean cortisol levels after one month of treatment with an

1058 antidepressant was 1.60 nmol/L (SE=0.40). The median was 0.80 nmol/L (95% CI
1059 0.81 - 2.34; range: 0.096-7.587) (Figure 1).

1060

1061 There was no statistically significant difference between the median cortisol levels
1062 at baseline and post antidepressant therapy (Wilcoxon match-pairs test:

1063 $W=380.50$; $p=0.857$)



1064

1065 (Error bars represent 1st and 3rd interquartile)

1066 **Figure 3.1: Median cortisol levels concentrations of the study population.**

1067

1068 **3.3 HAMD scores of the study population.**

1069 HAMD scores were collected on all 43 participants at the initial visit, prior to
1070 antidepressant therapy consumption. The HAMD scores ranged from 14 to 38. The
1071 mean score was 27.33; the median was 28.

1072

1073 For the participants who had cortisol levels, both a pre and post antidepressant
1074 therapy (n=28), the mean HAMD score at baseline was 28.36 (SE=0.92). The
1075 median HAMD score was 27.5 (95% CI 26.54 - 30.17; range: 16-38) (Figure 2).

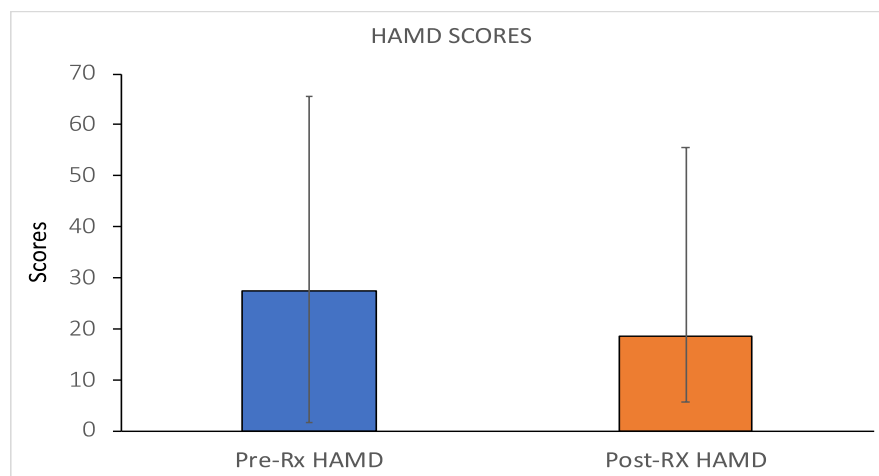
1076

1077 The mean HAMD score after one month of treatment with an antidepressant was
1078 18.93 (SE=1.44). The median HAMD score was 18.5 (95% CI 16.10 - 21.75; range:
1079 8-37) (Figure 2).

1080

1081 There was a significant difference between the median HAMD score at baseline and
1082 post antidepressant therapy (Wilcoxon match-pairs test: $W=119.00$; $p<0.001$).

1083 There was an overall reduction of 35% in the HAMD scores after antidepressant
1084 therapy.



1085

1086

(Error bars represent 1st and 3rd interquartile)

1087

Figure 3.2. Median HAMD scores of the study population.

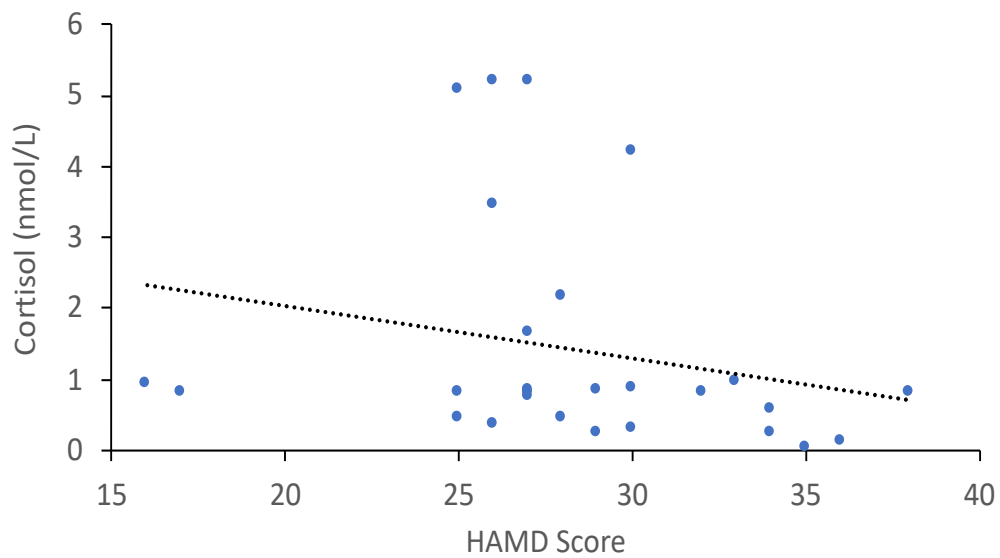
1088 **3.4 Correlation between HAMD scores and cortisol levels of the study**
1089 **population at baseline and after treatment with an antidepressant.**

1090

1091 There was a significant negative correlation between HAMD scores and cortisol
1092 levels at baseline (Spearman $r=-0.38$; $p=0.048$) (Figure 3).

1093

1094



1095

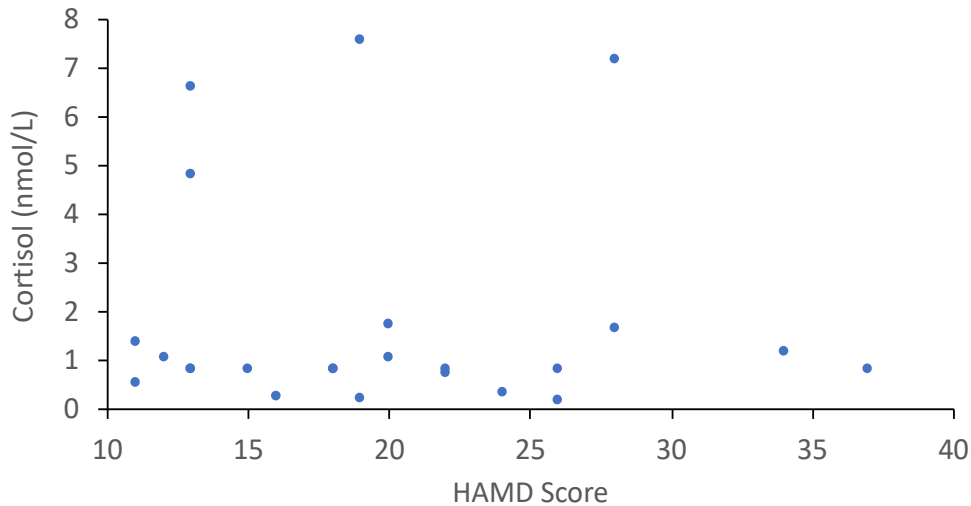
1096 **Figure 3.3. Correlation between HAMD scores and cortisol levels at baseline.**

1097

1098

1099 There was no correlation between the HAMD scores, and the cortisol levels post
1100 one-month treatment with an antidepressant (Spearman $r=0.16$; $p=0.422$) (Figure
1101 4).

1102



1103

1104 **Figure 3.4. Correlation between HAMD scores and cortisol levels post one-month**
1105 **treatment with an antidepressant**

1106

1107

1108 **3.5 Comparisons between median HAMD scores (at baseline and after**
1109 **one month of treatment) and socio-demographic characteristics of the**
1110 **study population.**

1111

1112 There were no statistically significant associations between the median HAMD
1113 scores at baseline and age ($H=1.41$; $p=0.494$); gender ($U=19$; $p=0.532$), race (U
1114 $=25.5$; $p=0.373$), habitation ($U=41.00$; $p=0.646$), schooling level ($U=42.00$;
1115 $p=0.673$), current employment ($U=90.50$; $p=0.888$) and family history of mental
1116 illness ($U=50.00$; $p=0.370$) in the (Table 2).

1117

1118

1119

1120

1121 **Table 3.2. Comparisons between baseline median HAMD scores and socio-**
 1122 **demographic characteristics.**

1123

| 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 |

1124

1125 There were no statistically significant associations between the median HAMD
 1126 scores after one month of treatment with an antidepressant and age (H=0.58;
 1127 p=0.749); gender (U=17.00; p=0.422), habitation (U=43.00; p=0.743), schooling
 1128 level (U=45.50; p=0.870), current employment (U=88.00; p=0.796), family history
 1129 of mental illness (U=66.00; p=1.00). However, the median HAMD scores were
 1130 significantly lower in Black compared to the Coloured participants after one month
 1131 of treatment (U=8.00; p=0.028) (Table 3).

1132

1133 **Table 3.3: Comparisons between median HAMD scores after one month of**
 1134 **treatment with an antidepressant and socio-demographic characteristics.**

1135
 1136

| 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 |

1137

1138

1139 **3.6 Comparisons between median cortisol levels (at baseline and after**
 1140 **one month of treatment) and socio-demographic characteristics.**

1141

1142 There were no statistically significant associations between the median cortisol
 1143 levels at baseline and age (H=2.55; p=0.280); gender (U=10.50; p=0.167), race
 1144 (U=31.0; p=0.629), habitation (U=29.00; p=0.212), schooling level (U=47.50;
 1145 p=0.974), and current employment (U=93.00; p=0.981) (Table 4). However,
 1146 participants with a family history of mental illness had significantly higher cortisol
 1147 levels compared to those who did not report a family history of mental illness
 1148 (U=24.00; p=0.019) (Table 4).

1149 **Table 3.4. Comparisons between baseline median cortisol levels and socio-**
 1150 **demographic characteristics.**

1151

| 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 |

1152

1153 There were no statistically significant associations between the median cortisol
 1154 levels after one month of treatment with an antidepressant and age ($H=0.36$;
 1155 $p=0.837$); gender ($U=25.00$; $p=0.929$), race ($U=25.00$; $p=0.929$), habitation
 1156 ($U=47.00$; $p=0.948$), schooling level ($U=37.50$; $p=0.491$), current employment
 1157 ($U=62.50$; $p=0.145$) and family history of mental illness ($U=65.00$; $p=0.955$) (Table
 1158 5).

1159

1160 **Table 3.5. Comparisons between median cortisol levels after one-month**
 1161 **treatment with an antidepressant. and socio-demographic characteristics**

1162

| 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 | $U = 65.00$; $p = 0.955$ |

1163

1164 **3.7 Comparisons between the change in median HAMD scores (from**
1165 **baseline to after one month of treatment) and socio-demographic**
1166 **characteristics.**

1167

1168 There were no statistically significant difference between the change in median
1169 HAMD scores (from baseline to after one month of treatment) and age groups
1170 (H=3.36; p=0.186), gender (U=16.00; p=0.372), race (U=13.50; p=0.08), marital
1171 status (U=41.50; p=0.700), level of education (U=40.0; p=0.600), employment
1172 (U=89.50 p=0.851), and family history of mental illness (U=55.00; p=0.538) (Table
1173 6).

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1187 **Table 3.6. Changes in median HAMD scores pre- and post- antidepressant**
 1188 **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 |

1189
 1190 It was noted that the change in HAMD scores was much lower in the 18-34 year
 1191 age group (-7) compared to the 35-50 year (-12) and the 51-65 year age group (-
 1192 12); in females (-9.0) compared to males (-16.0); in the coloured race (0.0)
 1193 compared to the black African race (-9.0); in married participants (-8.0) compared
 1194 to single participants (-9.5); in those with tertiary (-8.5) compared to secondary
 1195 schooling level (-10.0); in those currently unemployed (-8.0) compared to those

1196 employed (-10.0) and in those with a family history of mental illness (-7.0)
1197 compared to those without (-9.0) (Table 6).

1198

1199 **3.8 Comparisons between the change in median cortisol levels (from**
1200 **baseline and after one month of treatment) and socio-demographic**
1201 **characteristics**

1202

1203 It was noted that the change in cortisol levels was lower in the 18-34 year (-0.06)
1204 compared to 51-65 year age group (-0.03) and 35-50 year age group (-0.29); in
1205 females (-0.07) compared to males (-0.55); in the black African race (-0.06)
1206 compared to coloured race (0.61); in single participants (-0.07) compared to
1207 married participants (0.34); in those with secondary schooling level (-0.05)
1208 compared to tertiary schooling level (0.27); in those currently unemployed (0.13)
1209 compared to those employed (-16.0), and in those without a family history of
1210 mental illness (0.05) compared to those with a family history of mental illness (-
1211 0.51) (Table 7).

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1219 **Table 3.7. Changes in median cortisol levels pre- and post- antidepressant**
 1220 **treatment and socio-demographic characteristics.**

1221

| 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 |

1222

1223

1224

1225 **3.9 Comparisons between the antidepressant prescribed and change**
 1226 **in median HAMD scores and cortisol levels from baseline to post-**
 1227 **treatment.**

1228
 1229 Of the 28 participants who completed the study, 24 participants (85.7%) were
 1230 prescribed citalopram (dose 10mg daily {n=5} and dose 20mg daily {n=19}). Three
 1231 (10.7%) participants were prescribed fluoxetine (dose 20mg daily) and one (3.57%)
 1232 on another antidepressant (other than citalopram or fluoxetine).

1233
 1234 There were no statistical associations between the changes in median HAMD
 1235 scores (U=31.50; p=0.728) nor changes in median cortisol levels (U=29.00;
 1236 p=0.589) and the type of antidepressant used (Table 8). There was a significant
 1237 association between 10mg dosage of citalopram compared to the higher dosage of
 1238 20mg and the changes in median cortisol levels (U=20.0; p=0.050) but not for the
 1239 change in median HAMD scores (U = 47.50; p = 1.000) (Table 8).

1240

1241 **Table 3.8. Comparisons between changes in median HAMD scores and**
 1242 **median cortisol levels and the type of anti-depressants prescribed.**

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

1243

1244

1245 **4.Discussion**

1246

1247 **4.1 Cortisol levels**

1248

1249 *a) Prior to treatment with antidepressants*

1250

1251 The HPA axis and cortisol level dysregulation is reported to play a significant role
1252 in the pathophysiology of depressive disorders (Holsboer, 1996; Nemeroff,
1253 2005). This study found lower than normal levels of cortisol (hypocortisolism) in
1254 depressed participants prior to antidepressant therapy. A similar finding of
1255 hypocortisolism in depressed patients has been noted in other studies. Yehuda
1256 (1995) reported hyposecretion of cortisol in individuals who lived under conditions
1257 of ongoing or chronic stress. Oldehinkel (2001) also noted lower cortisol levels in
1258 persons with a chronic history (>2 years) of stress and depression. Bremmer
1259 (2007), referred to the concept of HPA axis exhaustion and low cortisol secretion,
1260 which results from chronic stress-related to psychological trauma, prolonged
1261 periods of work stress, or chronic low-grade immune activation. Penninx (2007)
1262 and Bremmer (2007) described a 'U-shaped' association between plasma cortisol
1263 (patient's plasma cortisol varying from normal to hypo- or hypercortisolaemia) and
1264 major depression in later life. Hypocortisolaemia occurred more commonly in the
1265 elderly subgroup and the authors associated this with physical frailty which was
1266 related to chronic exhaustion of the HPA axis and insufficient cortisol response to
1267 stressors.

1268

1269 This study's results are contrary to many studies, which found evidence of
1270 hyperactivity of the HPA axis (as indicated by higher daytime cortisol
1271 levels) among persons with depression (Sachar, 1973; Carroll, 1976; Pfohl,
1272 1985; Stokes, 1984; Gold, 1995; Bhagwagar, 2005). Otte (2004), reported
1273 baseline cortisol levels of 4.2 nmol/L in participants presenting with depression
1274 compared to 3.6 nmol/L in those with no depression. Khan (2019) also found
1275 increased cortisol levels of 2.2 nmol/L in patients with depression compared to

1276 1.46 nmol/L in healthy subjects ($p=0.031$). Vreeburg (2009), reported much higher
1277 average cortisol levels of 21.51 nmol/L in participants with major depressive
1278 disorder. When these results were extrapolated by Manthey (2011), they showed
1279 an average cortisol level of 15.7 nmol/L. Similarly, Shabaan (2015) also showed
1280 significantly higher cortisol levels amongst patients with depression (14.2 nmol/L)
1281 compared to healthy controls (11.5 nmol/L) ($p<0.01$). It is evident that despite the
1282 wide variation in the actual level of cortisol, many studies report elevated cortisol
1283 levels in depression compared to normal healthy individuals.

1284

1285 Chronic stress was considered as a possible explanation to account for the
1286 hypocortisolaemia finding in this study. Although longstanding stress and duration
1287 of depressive illness were not explicitly measured in this study, the low median
1288 cortisol level amongst the participants is likely a consequence of a community
1289 suffering from a high burden of chronic stress and social stigma. Chronic and
1290 ongoing stress is a common occurrence in South African. Many individuals are
1291 exposed to the risk factors of depression, such as lower educational attainment,
1292 poverty, poor access to adequate health care, lack of stable marriage and
1293 unemployment (Hamad, 2007). These individuals have higher perceived stress
1294 and have limited support from their community, as there is still an ongoing stigma
1295 towards mental illness (Hamad, 2007). The majority of the participants in our study
1296 were either single, separated or divorced, resulting in an impoverished support
1297 base (Ranga, 2019) or unemployed, which is associated with financial instability
1298 (Ranga, 2019). As the participants in our study had a younger profile (not
1299 consistent with that of Penninx (2007)), it is assumed that the hypocortisolism
1300 finding in our study was a result of the exhaustion of the HPA axis from chronic
1301 stress rather than physical frailty. It is recommended that future research should
1302 include a measure of longstanding stress or duration of depressive illness into
1303 their methodology, especially in a South African context. Our study also found that
1304 females were at a higher risk for chronic stress and hypocortisolism, which was
1305 similar to the findings of Bremmer (2007). It is also recommended that health care
1306 workers should be made aware of this and increase screening measures and early
1307 detection of depression in this high-risk group.

1308

1309 It is also possible that the low levels of cortisol could be attributed to
1310 methodological factors in this study, such as the time that the cortisol samples
1311 were collected and the waking times of the participants. In a meta-analysis by
1312 Miller (2007), which looked at the correlation between chronic stress and HPA
1313 axis, it was found that cortisol levels are usually elevated when the cortisol
1314 samples are acquired in the early morning and lowest in the evening. Laudat
1315 (1988) found that cortisol levels were the highest in the early hours of the morning
1316 between 8 to 9 am and decreases through the day with the nadir at around 11 pm.
1317 Nieman (2017b) reported that salivary cortisol concentrations vary diurnally, with
1318 levels around 15.4 nmol/L at 8 to 9 am and about 2.8 nmol/L at 11 pm.

1319

1320 In this study, the cortisol samples were collected on the same day of participants'
1321 appointment with their treating psychiatrist. This method was adopted after
1322 consideration of the participants' social circumstances and to minimize travel
1323 costs. As a result of this method, cortisol samples were collected any time
1324 between 8 am to 12 pm (average was approximately 11 am) after the participants
1325 had finished their consultation with their psychiatrist (this included the waiting time for
1326 file collection, nurses review, psychiatrist appointment and possibly waiting time to
1327 collect medication). Thus, the cortisol levels at this time of day would be well past
1328 the peak levels of 8-9 am.

1329

1330 Further, Seth (2016) reported that highest cortisol concentrations are detected
1331 about 30-45 minutes after waking. Many of the studies cited were performed in
1332 inpatient settings, and the cortisol samples were collected soon after waking. This
1333 study, however, was conducted in an outpatient clinic. Kane (2006) reported that
1334 the majority of patients utilising public health care facilities in South Africa use
1335 public transportation as they do not own cars. Therefore, for patients to arrive
1336 timeously for their clinic appointments, their waking times would most likely be
1337 very early.

1338

1339 The combination of early waking times and the late collecting times of samples
1340 could be significant contributors to the findings of low cortisol levels. Further
1341 research, in a South African setting, should be done in an inpatient setting to get a

1342 better understanding of the effect of the method of sampling on the results
1343 obtained.

1344

1345 *b) Post-treatment with antidepressants*

1346

1347 Our study found that the median cortisol levels after one month of treatment with
1348 an antidepressant remained unchanged. Similarly, Keating (2013) also reported
1349 relatively unchanged cortisol levels after SSRI therapy (88.3 ng/ml \pm 6.8 before and
1350 84.3 ng/ml \pm 6.2 after SSRI). Keating (2013) hypothesized that this may be
1351 attributed to only collecting a single cortisol sample (as was done in our study).
1352 They suggest that they might have shown a change in cortisol concentrations if
1353 there were multiple samplings done throughout the day, as individuals with
1354 depression often demonstrate an abnormal diurnal rhythm of cortisol.

1355

1356 In addition to the cortisol levels remaining unchanged following treatment, the
1357 levels in this study were also below normal. Hypocortisolism resulting from
1358 depression has been reported previously; however, in the majority of the literature,
1359 there is HPA axis hyperactivity which normalises with antidepressant therapy
1360 (Kapitany, 1999, Dziurkowska, 2013, Manthey, 2011). Antidepressants help in
1361 regulating the HPA axis by intensifying the feedback loop inhibition, thus
1362 decreasing the HPA axis hyperactivity in depressed patients (Pariante, 2008).
1363 Kapitany (1999), showed that cortisol levels decreased in depressed patients, after
1364 a 2-day citalopram stimulation procedure (from 9.4 mg dl to 8.8 mg dl). Likewise,
1365 Dziurkowska (2013) illustrated salivary cortisol suppression from 45.76 \pm 36.37
1366 ng/mL to 18.30 \pm 39.99 ng/mL after SSRI therapy. This improvement had also been
1367 reproduced by Manthey (2011), who described a reduction in the average cortisol
1368 level post antidepressant therapy. Manthey (2011) further differentiated cortisol
1369 level improvements according to the antidepressant treatment prescribed. Those
1370 using SSRI antidepressant therapy had an average of 15.8nmol/L (26.5%
1371 reduction), and TCA antidepressant therapy had an average of 18.3nmol/L (14.9%
1372 reduction) after one month of treatment initiation. Laakmann (2004), also reported
1373 normalisation of a hyperactive HPA axis with mirtazapine administration. They
1374 found the average cortisol level was 85.78 \pm 37.16 nmol/L per hour before
1375 mirtazapine administration in depressed patients. This cortisol level reduced after

1376 the administration of mirtazapine to an average 53.95 ± 14.79 nmol/L per hour by
1377 day 21 (37.1% reduction in cortisol levels). They concluded that mirtazapine had
1378 acutely inhibit cortisol secretion and resulted in normalisation of cortisol
1379 levels. Nandam (2020) found normalisation of cortisol levels with chronic
1380 antidepressant use. They described findings of reduced urinary cortisol levels after
1381 fluoxetine treatment after four months. Holsboer (1996), also described the clinical
1382 effectiveness of antidepressant therapy by looking at longitudinal studies of
1383 dexamethasone suppression studies. They noted a normal response to
1384 dexamethasone suppression after antidepressant use. Aihara (2007)
1385 demonstrated HPA axis normalisation in a group of depressed participants post
1386 antidepressant therapy.

1387

1388 Furthermore, studies, where an initial hypocortisolism at baseline was found, have
1389 reported a later cortisol normalization after antidepressant therapy (Murck (2003)).
1390 The type of depression in this subgroup was termed atypical depression as they
1391 had reversed vegetative signs. This group were prescribed monoamine oxidase
1392 inhibitors (MAOI) and hypericum extract, which lead to an activation of HPA axis
1393 activity and thus normalisation of the HPA axis. This absence of normalisation in
1394 our study could be attributed to the use of SSRI and not MAOI as used in Murck
1395 (2003). MAOIs are not routinely used in South Africa as a first-line agent due to
1396 potential adverse effects (The South African Society of Psychiatrists treatment
1397 guidelines for psychiatric disorders, SASOP, 2013).

1398

1399 However, Dziurkowska (2013) reported in their study, that after utilisation of SSRIs
1400 (such as sertraline, citalopram, escitalopram, and paroxetine) that there was an
1401 increase in cortisol levels around day 30 (but lower than the initial pre-treatment
1402 cortisol level) with a subsequent decrease thereafter. It is possible that the failure
1403 of this study to show HPA axis normalization after SSRI treatment could be due to
1404 the timing of the cortisol collection (cortisol sampling was done at day 28) as
1405 compared to later in the above study. Another factor was that Dziurkowska (2013)
1406 used participants from Poland, which is a more developed country and with
1407 different socioeconomic considerations compared to a South African population
1408 sample.

1409 These studies show an improvement with antidepressants, differentiating them
1410 from this study. It is essential to consider that there were many methodological
1411 differences such as duration of the studies (an extended period showed a more
1412 considerable improvement in cortisol levels) and type of intervention used
1413 (different antidepressant therapies were prescribed). Similar further research
1414 should be conducted, perhaps with an extension of the duration of the study, as
1415 well as, to assess the difference in response to various antidepressant treatments
1416 in a South African context.

1417

1418 Additional factors accounting for low cortisol levels before the initiation of
1419 antidepressants include the late salivary cortisol collection times and early waking
1420 times of participants as discussed above.

1421

1422

1423 *c) Associations between the change in cortisol levels pre and post-*
1424 *treatment and other variables*

1425

1426 This study found no significant change in the cortisol levels pre and post-treatment
1427 with an antidepressant. Nor any significant association with any variables and the
1428 overall change in median cortisol levels pre and post-treatment.

1429

1430 Many studies describe the association between change in cortisol levels pre and
1431 post antidepressant therapy. There is, however, a dearth of knowledge regarding
1432 the association between the change in cortisol levels and other variables pre and
1433 post antidepressant therapy. Majority of studies have collected sociodemographic
1434 data of their participants but have not further extrapolated its association with
1435 cortisol levels. In our context, the sociodemographic data may indicate the
1436 presence of chronic stress; hence other studies demonstrating that association
1437 would be beneficial to this study.

1438

1439 Keating (2013) reported no change in cortisol levels pre and post antidepressant
1440 (SSRI) therapy and found no difference in response between males and females
1441 after comparing the cortisol levels. Similarly, this study showed no association
1442 between gender and change in cortisol levels.

1443 **4.2 HAMD scores**

1444

1445 *a) Prior to treatment with antidepressants*

1446

1447 This study found a median HAMD score at entry to be 27.5, which is above the
1448 cut-off score for severe depression (≥ 23). Similarly, in other studies, high HAMD
1449 scores in depressed patients prior to treatment have been reported. Zhou (2017),
1450 in a study of 596 participants (299 were diagnosed with MDD, and 297 were the
1451 control group) showed an average HAMD score of 23.8 before treatment. Young
1452 (2004), found a response to fluoxetine in 20 treatment naïve participants with
1453 MDD, who reported an average HAMD score of 24 ± 3 at entry to study. Yehuda
1454 (1996) compared cortisol regulation in PTSD and MDD participants and reported a
1455 HAMD score of 29.46 ± 7.3 in the male, treatment naïve cohort group diagnosed
1456 with MDD. Gou (2015) studied 120 female patients diagnosed with MDD and
1457 reported an average HAMD scores of 22.4 before any antidepressant
1458 intervention.

1459

1460 This study found higher average HAMD scores compared to the majority of the
1461 above studies, which could suggest a late presentation to health care
1462 establishments. Thus, strategies targeting high-risk patients in primary care
1463 centres should be a crucial factor in protocol development.

1464

1465 *b) Post-treatment with antidepressants*

1466

1467 It must be noted that an improvement in HAMD score does not always equate to
1468 adequate response/resolution of MDD. Only those with a $>50\%$ improvement in
1469 HAMD scores are labelled as responders to antidepressant therapy. This study
1470 was able to show a statistically significant improvement in the median HAMD
1471 score (35%) after treatment with SSRI antidepressant therapy. While this study
1472 showed an improvement in average HAMD scores, it was not enough to mark the
1473 entire group as responders after one month of antidepressant treatment. Many of
1474 the participants still described symptoms of their depression which would place
1475 them in the category of moderate depression.

1476

1477 Nikkheslat (2019) also reported a variable reduction in HAMD scores in their
1478 sample group after six weeks of antidepressant therapy. They conducted a cross-
1479 sectional study dividing participants into three categories depending on the
1480 response to antidepressant therapy (responders, non-responders and those not
1481 yet on treatment). The average HAMD scores were as follows: untreated
1482 depression (20.3), non-responders (18.1) and responders (3.6). Approximately two
1483 times more participants were non-responders compared to responders. Similarly,
1484 this study found more non-responders compared to responders after
1485 antidepressant therapy.

1486

1487 One reason for the suboptimal response to antidepressants could be due to the
1488 short duration of this study. However, this inadequate response to antidepressants
1489 goes against the findings found by Szegedi (2009), who demonstrated that early
1490 improvement (less than two weeks) with antidepressant therapy using the HAMD,
1491 was a good predictor of stable response and remission. Another reason for partial
1492 response to treatment may be because no other form of intervention such as
1493 psychotherapy or social worker support was offered as an adjunct to
1494 pharmacotherapy. The participants' self-perceived lack of change/ improvement in
1495 their social or financial problems could have impacted on their response to
1496 pharmacotherapy. Both low support (Patten, 2010) and inadequate economic
1497 resources (Bracke, 2013) are risk factors for depression. A South African
1498 community with limited socioeconomic resources likely requires added support in
1499 the form of support groups, individual therapy and social worker intervention in
1500 addition to pharmacotherapy to manage their depression adequately. Another
1501 reason for the suboptimal response to antidepressants was the possible
1502 misdiagnosis of the participants. There could have been a missed personality
1503 disorder or bipolar depression. Their coping skills were also not assessed. All of
1504 these could have attributed to the suboptimal response noted in this study.

1505

1506 This study is in contrast to other studies where participants showed an adequate
1507 response to treatment (HAMD improvement by at least 50%). Studies such as
1508 Young (2004), indicate an improvement in HAMD scores from a baseline of 24 ± 3
1509 (66.67% reduction) to 8 ± 2.7 , in the treatment responder group, after six weeks of
1510 fluoxetine. Similarly, Tadić (2010) showed improvement in depression

1511 (improvement of HAMD scores) after other subtypes of antidepressant therapy
1512 were commenced. Tadić (2010) assessed the response of 84 depressed
1513 participants, with baseline HAMD scores of 16.9 ± 4.5 , after sertraline
1514 administration. After one month of sertraline antidepressant therapy, 60% of the
1515 participants showed a greater than 20% decrease in HAMD score and 15% had
1516 greater than 50% decrease in HAMD score (actual HAMD scores post
1517 antidepressant therapy were not provided).

1518
1519

1520 *c) Associations between change in HAMD scores and other variables*

1521

1522 There was no significant association between the overall change in HAMD scores
1523 pre and post-treatment with most demographic and clinical variables. It was noted
1524 that median HAMD scores were lower in Black African participants compared to
1525 the Coloured participants after one month of antidepressant therapy. The only
1526 variable known to have changed in this sample group was the addition of
1527 antidepressant treatment. Therefore, it could be extrapolated that the difference in
1528 response rate amongst the two racial groups would be their sensitivity to SSRI
1529 antidepressant therapy.

1530

1531 Others have investigated remission rates of depression amongst different racial
1532 groups. Lesser (2007) found no statistical significance amongst different racial
1533 groups and HAMD scores but did find lower remission rates amongst Black
1534 American participants compared to White participants and Hispanic participants.
1535 This finding was also noted by Fortuna (2010), who showed poor treatment
1536 retention and thus reduced remission rates of depression amongst the ethnic and
1537 racial minority in the United States of America. Poor treatment retention in African
1538 American patients was attributed to superficial disclosing of information to health
1539 care workers, abrupt termination of counselling, their belief that the mental illness
1540 would improve by itself and the lack of racial concordance between patient and
1541 health care provider. Further research in a South African setting would need to be
1542 done to assess poor treatment retention. A similar discordance between health
1543 care provider and participant likely occurs in a South African context, which may
1544 attribute to the participants having a reduced remission rate.

1545

1546 This study also showed no statistically significant associations between the
1547 changes in median HAMD scores and the type of antidepressant used. Both
1548 treatment options were in the same antidepressant class (SSRI). Therefore, this
1549 could be a reason that there was no difference in efficacy between citalopram and
1550 fluoxetine.

1551

1552 In the South African public sector, there are limited available treatment options,
1553 with SSRIs being the first line of treatment (The South African Society of
1554 Psychiatrists treatment guidelines for psychiatric disorders, 2013). The increased
1555 use of SSRIs would only be a quandary if the efficacy of SSRI were suboptimal as
1556 compared to other antidepressant agents. Geddes (2007) analysed 98
1557 randomised control studies to assess the effectiveness of SSRI treatment as well
1558 as compare SSRI treatment with the older tricyclic antidepressants (TCA). Geddes
1559 (2007) found no clinically significant differences in efficacy between SSRIs and
1560 TCA; instead, treatment choice should be based on patient preference, drug
1561 toxicity and cost. Similarly, Hirschfeld (1999) reviewed three meta-analysis and 20
1562 clinical trials and found that SSRIs had comparable efficacy to TCA for the
1563 treatment of severe or melancholic depression. Patris (1996) performed a double-
1564 blind, randomized trial comparing citalopram and fluoxetine in patients with
1565 unipolar depression. They found a reduction in the HAMD score in both groups
1566 with no statistically significant differences between treatments, including adverse
1567 effects. These studies show similar efficacy between SSRIs and TCA; thus, the
1568 treatment options used are justified.

1569

1570 There was no significant association between the 10mg dosage of citalopram
1571 compared to the higher dosage of 20mg and the changes in median HAMD
1572 scores. No significant association between the dosages could be attributed to
1573 impaired tolerance of medication at a higher dosage; and the antidepressant effect
1574 of citalopram is still present with the 10mg dose (Bech, 2002). Torta (2012)
1575 describes that a patient's motivation and adherence is linked to the tolerability of
1576 treatment. Therefore, it is essential to look at both the side effect profile of
1577 antidepressant therapy at higher doses as well as the clinical response with lower
1578 doses. Torta (2012) analysed studies from colleagues, many from Italy (a first

1579 world country); a nation of increased resources (possibly psychotherapy)
1580 compared to South Africa. Increased resources afford health care workers to
1581 adjunct antidepressant (e.g. with psychotherapy) in their participants. Therefore,
1582 health care workers would have the advantage of slow up-titration of the
1583 antidepressants. In a South African setting, where there are limited resources, a
1584 health care worker may not have the luxury of slow up-titration.

1585

1586 **4.3 Correlations between HAMD scores and cortisol levels**

1587

1588 This study found a significant inverse relationship between cortisol and HAMD
1589 scores at baseline; as the HAMD scores increased, the cortisol levels decreased.
1590 This inverse relationship is in keeping with multiple studies that suggest that
1591 chronic stress leads to hypocortisolaemia. Such as described by Oldehinkel
1592 (2001), who noted that low cortisol levels were present in those with chronic
1593 depression (>2 years). Penninx (2007) also described hypocortisolaemia in the
1594 elderly subgroup, which they attributed to physical frailty. The inverse relationship
1595 between cortisol and HAMD scores were also noted by Bremner (2007), who
1596 explored HPA axis exhaustion from chronic stress resulting in hypocortisolaemia.

1597

1598 This study's findings are, however, are in contrast with some studies where a
1599 positive correlation was noted. Studies have shown an association between
1600 HAMD scores and cortisol by illustrating hypercortisolaemia with MDD (high
1601 HAMD scores). Maes (1986) demonstrated elevated cortisol levels in 100
1602 depressed patients post dexamethasone suppression test at baseline, thus
1603 showing a positive correlation between cortisol levels and HAMD scores. They
1604 further stratified their participants into major and mild depression based on the
1605 severity of elevation of their cortisol level. Participants with major depression had
1606 both higher HAMD scores as well as cortisol values compared to those who had
1607 minor depression. The highest cortisol levels, at the various measurement times (8
1608 am, 4 pm and 11 pm), in the participants were the best predictors for severity of
1609 the depression when measured with the HAMD score. Yoon (2015) showed that
1610 there were significant positive correlations between plasma cortisol levels and the

1611 depression component of the HAMD and vice versa; where cortisol levels rose
1612 with the severity of depression (as shown by increasing HAMD scores).

1613

1614 There was no correlation between the HAMD scores and the cortisol levels one-
1615 month post-treatment with an antidepressant. This lack of an association could be
1616 explained on the basis that our patients already had hypocortisolaemia at
1617 baseline. On the other hand, other studies which have shown a normalisation of
1618 the HPA axis after antidepressant therapy. Studies such as Laakmann (2004),
1619 showed elevated cortisol levels in 12 depressed patients, which improved with
1620 resolution of depression. Similarly, Kapitany (1999), showed a reduction in cortisol
1621 levels (from 9.4 mg dl to 8.8 mg dl) in 16 patients diagnosed with acute major
1622 depression after citalopram 20 mg, 2-day stimulation procedure; the participants in
1623 this trial had HAMD scores ranging from 19-32, but due to the short trial, HAMD
1624 scores were not repeated. Likewise, Navines (2007), studied a group of
1625 participants with MDD (HAMD score >17, mean HAMD score 22.2) and
1626 demonstrated a decrease in HPA axis overactivity after administration of
1627 citalopram for eight weeks; these patients showed a reduction in ACTH, cortisol
1628 and HAMD score (mean 11.48).

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1639 **5. Limitations**

1640 The following limitations of this study are noted:

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1642 1) For meaningful statistical analysis, the sample size was determined to be 51
1643 patients. However, only 28 patients completed the study because a significant
1644 number failed to keep their one month follow up appointment and had to be
1645 labelled as dropouts.

1646 2) Recruitment was difficult as large numbers of patients referred for
1647 participation in the study had to be excluded. Some were not antidepressant
1648 therapy naïve at baseline (treatment already initiated by primary health care
1649 professionals), and others had co-morbid medical condition or another axis I
1650 psychiatric diagnosis (which is common in this population).

1651 3) Salivary cortisol samples should have been collected at peak cortisol
1652 secretion based on circadian rhythm, which is 30-40 minutes after waking.
1653 Because of the outpatient nature of this study and the time taken for patients
1654 to have their samples collected, it ended up being many hours after waking
1655 and may have impacted on the cortisol levels recorded.

1656 4) To assess the HPA axis response to antidepressants, patients needed to be
1657 adherent to their medication. There is no objective test that can reliably
1658 ascertain adherence to antidepressants at our clinics; hence the researchers
1659 relied on a pill count at the second visit. This method is unreliable and subject
1660 to manipulation by patients to appease the doctor.

1661 5) The validity of the HAMD in this sample population may be affected by the
1662 fact that this was a predominantly African population subgroup, with English
1663 not being their first language. As such, there may have been a
1664 misunderstanding of some of the English nuances in the HAMD, resulting in
1665 misinterpretation and thus affecting the grading scales.

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1671 **6. Recommendations**

1672 1) It is recommended that further studies be done in this area with larger sample
1673 size, including variables such as participants' waking times, grading stress
1674 levels and childhood trauma, objective monitoring of treatment adherence,
1675 and longer duration to allow more time for potential cortisol normalization post
1676 antidepressant therapy. It would also be advantageous that future studies
1677 utilise a validated rating scales in the participant's home language.

1678
1679 2) While this study showed an improvement in average HAMD scores, it was not
1680 enough to mark the entire group as responders after one month of
1681 antidepressant treatment. Many of the participants still described symptoms of
1682 their depression which would place them in the category of moderate
1683 depression. In addition, this study found that the participants had low cortisol
1684 levels at entry to study, suggesting that they may be experiencing chronic
1685 stress which was contributing to their depression. It is therefore
1686 recommended that mental health service providers ensure that all depressed
1687 patients be provided with a more holistic management, including
1688 psychotherapy and social worker intervention to facilitate a change in the
1689 patient's home life.

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1704 **7. Conclusion**

1705 There is limited research on cortisol activity amongst depressed South Africans as
1706 well as the response with an antidepressant on normalising cortisol levels and
1707 improving depressive symptoms. This study illustrated a significant improvement
1708 in the severity of depression in the participants after one-month of antidepressant
1709 treatment. This was evidenced by improved HAMD scores. The authors are of the
1710 opinion that the patients would have yielded much better responses had the
1711 treatment been for a longer duration and included adjunctive therapy such as
1712 psychotherapy and social worker intervention.

1713

1714 However, this study did not find high circulating cortisol due to HPA axis
1715 dysregulation amongst the depressed patients, but rather hypocortisolaemia. It is
1716 postulated that, similar to other studies which reported these findings, the low
1717 cortisol levels may be attributed to chronic stress experienced by the participants
1718 as a result of their lower socioeconomic and disadvantaged backgrounds.

1719 Treatment with an antidepressant had no impact on the cortisol levels. Hence,
1720 cortisol levels in this population is not always useful as a predictor of the severity
1721 of depression or serves as a measure of the response to treatment.

1722

1723 It is recommended that further studies be conducted in a larger sample group and
1724 for a longer duration to investigate this population's baseline cortisol level and
1725 response to antidepressant therapy. The study should also look at the effect of
1726 severity and duration of social stressors on cortisol levels.

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