**Adrenal insufficiency associated with advanced HIV may explain the high mortality.**

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**Abstract:**

**Background:**

Sub-Saharan Africa is burdened by a vast HIV-positive population, at risk of life-threatening AIDS defining illnesses. AIDS defining illnesses and disseminated opportunistic infections have been associated with both primary adrenal insufficiency (PAI) and secondary adrenal insufficiency (SAI). We hypothesized that hypoadrenalism may partially account for the high mortality seen with advanced HIV.

**Materials & Methods:**

We undertook a prospective case-finding study of HIV-positive patients presenting with advanced disease who were 18 years or older, with a CD4 count of less than 100 cells per mm3, and a coexistent opportunistic infection. Exclusion criteria were use of oral, intravenous or inhaled steroids in the previous 3 months. A tetracosactide test was performed in patients with random cortisol concentrations in the morning of less than 500 nmol/L.

**Results:**

A total of 515 patients were recruited, and 431 complete records evaluated. The median and interquartile range (IQR) age of patients at enrolment was 36.0 (31.0-42.0) years. The majority of whom were black Africans (83.3%) and mixed-race (15.9%), followed by white and Asians constituting 0.5% and 0.2%, respectively. Of the 431 patients 31 (7.2%) had adrenal insufficiency (AI), of whom 24/31 (77.42%) had SAI, whereas 7/31 (22.58%) had PAI. Opportunistic infections (OI’S) were predominantly tuberculosis in 73%, cytomegalovirus (CMV) (0.2%) and *Cryptococcal neoformans* in 0.2%, respectively. Patients with AI versus those without, had marginally higher diastolic blood pressure 71 (70-80) mmHg vs 70 (60-78) mmHg (*p*=0.031), and lower potassium 3.70 (3.30-4.00) mmol/L versus 4.20 (3.70-4.60) mmol/L (*p*=0.045), respectively. Patients with AI demonstrated lower morning serum cortisol 332 (253-375) nmol/L versus 513 (388-606) nmol/L (*p*<0.001), reduced basal cortisol 300 (185-328) nmol/L versus 462 (352-568) nmol/L (*p*<0.001), and diminished stimulated cortisol 403 (316-438) nmol/L versus 720 (616 -848) nmol/L (*p*<0.001). The median random plasma ACTH 37 (25-72) pg/mL versus 31 (18-48) pg/mL (*p*=0.029) was higher in patients with AI. Overall, a total of 150 tetracosactide test were performed identifying 31 AI patients. There was a higher one-year mortality in the AI 17/31 (54.83%), compared with the non-AI groups 89/400 (22.25%); (*p*=0.0015), and the survivors continue their antiretroviral and cortisol replacement therapy.

**Conclusion:** Among a cohort in the largest study on AI in advanced HIV in Africa, AI occurred in 7.2% of the participants. Overall mortality was 24.59% and the AI group was 54.83% versus 22.25%: (*p*=0.0015) for the non-AI groups, respectively. Higher mortality in

patients with AI may reflect a more advanced state of ill health with reduced reserve. It is unknown at this stage whether the AI is transient or chronic.

**Introduction:**

Adrenal insufficiency is a potentially fatal medical condition, caused by deficiency of glucocorticoids and mineralocorticoids. Prior to the availability of glucocorticoid therapy, the majority of the primary adrenal insufficiency patients died within 2 years of diagnosis (1). Autoimmune adrenal insufficiency predominates in European populations, (2) and in South Africa, despite the high background prevalence of tuberculosis (TB) (3). Our recent survey suggests that coexistent infections are a significant cause of primary adrenal insufficiency in sub-Saharan Africa with an estimated prevalence of 34% and 29.8% for TB and Acquired Immune Deficiency Syndrome (AIDS), respectively (4).

Secondary adrenal insufficiency is predominantly caused by pituitary surgery, radiation, apoplexy, infections including TB and histoplasmosis, infiltrative diseases (sarcoidosis), tumors, trauma and large intracranial aneurism (6). AIDS has the potential to affect all organs either directly or indirectly via aids defining illnesses, including endocrine glands (7), leading to secondary hypogonadism mediated by cytokine interaction of the hypothalamus, adrenalitis and thyroid dysfunction (5).

There are conflicting data describing the incidence and prevalence of hypoadrenalism in patients with HIV. In a Pakistan study of 64 HIV infected, predominantly male patients (84.9%), Afreen *et al*, reported adrenal insufficiency (AI) in 9 (14.06%), using the of 250 microgram intravenous tetracosactide test and a 60 minutes cortisol concentration of less than 18 microgram/dl (500 nmol/L),(8) whereas in a Nigerian study of 43 newly diagnosed HIV positive patients who were treatment naïve patients, the reported AI incidence was 34.8%, using the 1 microgram tetracosactide test (9) and a 30minute cortisol of less than 500 nmol/L.

There is a high background prevalence 9% (10) of Human immune virus (HIV) infection (7) in sub-Saharan Africa, which is associated with significant mortality estimated in 2001 at 2.2 million out of 3 million deaths in patients presenting with advanced disease worldwide (12). Compared to Europe and North America, early mortality in HIV positive patients is higher in resource limited settings, including South Africa (12) due to late presentation and inadequate health systems. Although undiagnosed adrenal insufficiency is associated with invariable mortality (13), treated adrenal insufficiency is associated with poorer survival due in part to cardiovascular, malignant and infectious diseases, compared to the background population (14) (15), especially in overtreatment and insufficient replacement during infections and stress-related events.

On the background of substantial mortality associated with advanced HIV, we hypothesized that the additional diagnosis of adrenal insufficiency may exacerbate mortality. Moreover, we considered that initiation of appropriate replacement therapy for adrenal insufficiency may confer a positive impact on mortality. Our objectives were to determine the incidence of hypoadrenalism among ill, hospitalized HIV-infected patients. In addition, we wished to explore the positive predictive clinical and biochemical characteristics for adrenal insufficiency, and the predictors for survival. Additionally, we were interested in determining the extent to which adrenal insufficiency was transient or otherwise.

**Materials and methods:**

Permission to conduct the study (HREC 163/2015) was obtained from the University of Cape Town Faculty of Health Sciences, Human Research and Ethics Committee, which endorses the latest Helsinki declaration of 2013. We undertook a prospective case-finding of HIV positive patients, presenting with advanced disease and an opportunistic infection to an acute tertiary care medical ward. All the participants provided written informed consent. If participants were affected by delirium the research and ethics committee permitted us to take retrospective consent, with the view of limiting bias and providing an opportunity for all patients to receive potentially life-saving treatment.

*Inclusion criteria*

Inclusion criteria included age 18 years and older, and a CD4 count of 100 cells per mm3 or fewer and an opportunistic infection.

*Exclusion criteria*

The use of oral or inhaled steroids in the previous three months.

*Data extraction*

Demographic and clinical data were obtained from history and physical examination findings and a record of routine biochemistry, haematology and microbiology from the National Health Laboratory Service (NHLS) repository. Patients who met the inclusion criteria had blood samples taken for serum cortisol and plasma ACTH between 08:00 and 09:00. Where the random serum cortisol was less than 500 nmol/L, a short 250 µg intravenous tetracosactide stimulation test was performed, usually the following day.

*Biochemical measures of plasma ACTH and serum cortisol*

For the random plasma ACTH (Purple top on ice) and cortisol, clotted blood and plasma were submitted to the Lancet laboratory which analyzed the samples on the Cobas immunoassay platform (Biotinylated monoclonal anti-cortisol antibody)

*The tetracosactide test*

The samples for plasma ACTH and cortisol were submitted to the National Health Laboratory Services (NHLS). The method employed by this laboratory is the Roche (Modular Analytics E170):

Requirements

* 2 clotted gel tubes
* 250 microgram Synacthen (1 vial)

Procedure

|  |  |
| --- | --- |
|  | 09h00: 3 mL blood for cortisol, and inject Synacthen ivi. |
|  | 09h30: take further sample for cortisol. |

Interpretation

Adrenal insufficiency is excluded by a 30 min value > 550 nmol/L (Roche ().

*Interpretation of plasma cortisol and ACTH*

In circumstances where the random cortisol was less than 500 nmol/L, a short 250 µg tetracosactide test was performed intravenously with samples taken at 0 minutes and 30 minutes, respectively. Adrenal insufficiency was excluded if the 30-minute stimulated cortisol is > 500 nmol/L. Secondary adrenal insufficiency was diagnosed with a normal ACTH or less than 2.68 pg/mL and a 30 min cortisol < 500 nmol/L.

*Determination of survival*

Telephonic follow-up was done to determine post discharge survival via direct patient or family contact. For analysis we described those who were discharged from the hospital within 3 months (early survivors), followed by 6 months (intermediate survivors), and 12 months follow-up (late survivors).

**Statistical analysis:**

Categorical variables were presented as frequencies and percentages, and continuous variables were expressed as medians (16) and were compared using the Wilcoxon-Mann-Whitney test. Proportions and categorical variables were compared using Pearson’s chi-square test or Fisher’s exact test as appropriate. Univariate logistic regression was used to estimate the association between AI and various risk factors. Multivariable logistic regression analysis was used to identify independent predictors of AI. We ranked the participants into tertiles of CD4 counts: 0-30; 31-60 and 61-100 to determine if AI was associated. The statistical analysis was done using STATA Statistical Software version 15.The significance level was set at *p*<0.005.

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**Results**

**Clinical characteristics**

A total of 515 patients were recruited, 17 were unwilling to participate and 431 had complete data for analysis. The median age at enrollment was 36 years (IQR 31.0-42.0) overall, 35 and 37 years and for females and males, respectively. The majority were black Africans 356 (83%), followed by those of Mixed ancestry 68 (15.9%), White 2 (0.5%), and Asian 1 (0.2%). The median (IQR) duration of the presenting illness was 14 (14-21) days. The baseline clinical characteristics are presented in Table 1. Older age at presentation was associated with higher CD4 counts. Nausea, diarrhoea and postural drop predominated in the lowest tertile of CD4 count with significant p-values of *p*=0.013, *p*=0.020, and *p*=0.038, respectively. In respect of the investigations, the white blood cells, neutrophils and viral load predominated in the higher CD4 counts with p-values at *p*<0.0001, *p*=0.038, and *p*<0.001, respectively.

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| --- | --- | --- | --- | --- | --- | --- |
| Table 1: Patient demographic and clinical characteristics | | | | | | |
| Variable | *N* | Overall, *N* = 428 | 0 - 30, *N* = 210 | 31 - 60, *N* = 113 | 61 - 100, N = 105 | *p*-value |
| **Demographics** |  |  |  |  |  |  |
| **Age at enrolment** | 428 | 36 (31, 42) | 34 (30, 41) | 37 (32, 43) | 37 (32, 46) | **0.012** |
|  |  |  |  |  |  |  |
| **gender** | 427 |  |  |  |  | 0.3 |
| Females |  | 217 (50.8%) | 104 (49.8%) | 64 (56.6%) | 49 (46.7%) |  |
| Males |  | 210 (49.2%) | 105 (50.2%) | 49 (43.4%) | 56 (53.3%) |  |
| **Ethnicity** | 426 |  |  |  | 000 | 0.2 |
| Asian |  | 1 (0.2%) | 1 (0.5%) | 0 (0.0%) | 0 (0.0%) |  |
| Black African |  | 355 (83.3%) | 180 (86.1%) | 95 (84.1%) | 80 (76.9%) |  |
| Coloured |  | 68 (16.0%) | 27 (12.9%) | 17 (15.0%) | 24 (23.1%) |  |
| White |  | 2 (0.5%) | 1 (0.5%) | 1 (0.9%) | 0 (0.0%) |  |
| **History:** |  |  |  |  |  |  |
| **Duration of current illness** | 400 | 14 (14, 21) | 14 (14, 21) | 14 (14, 29) | 14 (8, 21) | 0.3 |
| **Fatigue** | 411 | 349 (84.9%) | 175 (86.2%) | 92 (83.6%) | 82 (83.7%) | 0.8 |
| **Weakness** | 410 | 343 (83.7%) | 170 (84.6%) | 94 (84.7%) | 79 (80.6%) | 0.6 |
| **Poor appetite** | 408 | 307 (75.2%) | 157 (78.1%) | 81 (73.6%) | 69 (71.1%) | 0.4 |
| **Weight loss** | 412 | 363 (88.1%) | 181 (89.2%) | 97 (87.4%) | 85 (86.7%) | 0.8 |
| **Nausea** | 410 | 209 (51.0%) | 115 (56.9%) | 56 (50.9%) | 38 (38.8%) | **0.013** |
| **Vomiting** | 409 | 112 (27.4%) | 57 (28.4%) | 29 (26.4%) | 26 (26.5%) | >0.9 |
| **Diarrhoea** | 407 | 162 (39.8%) | 93 (46.0%) | 41 (37.3%) | 28 (29.5%) | **0.020** |
| **Liking for salt** | 407 | 256 (62.9%) | 138 (68.7%) | 69 (63.3%) | 49 (50.5%) | 0.010 |
| **Dizziness** | 407 | 179 (44.0%) | 100 (49.8%) | 44 (40.0%) | 35 (36.5%) | 0.060 |
| **Loss of consciousness** | 407 | 5 (1.2%) | 3 (1.5%) | 1 (0.9%) | 1 (1.0%) | >0.9 |
| **Clinical findings** |  |  |  |  |  |  |
| **Hypoglycaemia** | 408 | 9 (2.2%) | 4 (2.0%) | 2 (1.8%) | 3 (3.1%) | 0.8 |
| **Hypotension** | 408 | 30 (7.4%) | 16 (8.0%) | 11 (10.1%) | 3 (3.1%) | 0.14 |
| **BP (systolic)** | 427 | 110 (100, 125) | 110 (100, 123) | 114 (100, 130) | 112 (105, 121) | 0.2 |
| **BP (diastolic)** | 427 | 70 (60, 78) | 70 (60, 80) | 70 (60, 79) | 69 (60, 75) | 0.7 |
| **Any postural drop in blood pressure** | 410 | 14 (3.4%) | 11 (5.4%) | 3 (2.7%) | 0 (0.0%) | **0.038** |
| **Shock** | 411 | 5 (1.2%) | 3 (1.5%) | 1 (0.9%) | 1 (1.0%) | >0.9 |
| **Anorexia** | 409 | 172 (42.1%) | 95 (47.3%) | 45 (40.9%) | 32 (32.7%) | 0.054 |
| **Loss of axillary and pubic hair, if female** | 414 |  |  |  |  | 0.14 |
| No |  | 151 (36.5%) | 67 (32.5%) | 50 (45.5%) | 34 (34.7%) |  |
| N ot applicable |  | 195 (47.1%) | 102 (49.5%) | 42 (38.2%) | 51 (52.0%) |  |
| Yes |  | 68 (16.4%) | 37 (18.0%) | 18 (16.4%) | 13 (13.3%) |  |
| **Increased skin pigmentation** | 395 | 180 (45.6%) | 98 (49.2%) | 46 (43.0%) | 36 (40.4%) | 0.3 |
| **Investigations** |  |  |  |  |  |  |
| **Presence of anaemia** | 406 | 223 (54.9%) | 119 (59.5%) | 59 (54.1%) | 45 (46.4%) | 0.10 |
| **Haemoglobin g/dL** | 425 | 8.70 (7.40, 10.30) | 8.60 (7.50, 9.90) | 8.70 (7.40, 9.90) | 9.20 (7.40, 11.05) | 0.10 |
| **Presence of an opportunistic infection** | 425 | 422 (99.3%) | 207 (99.5%) | 111 (99.1%) | 104 (99.0%) | >0.9 |
| **White cell count X109** | 422 | 5.3 (3.5, 8.0) | 4.9 (2.7, 6.8) | 5.8 (4.3, 9.0) | 6.6 (4.4, 9.7) | **<0.001** |
| **Lymphocyte count X109** | 93 | 0.8 (0.4, 1.8) | 0.6 (0.3, 1.4) | 0.9 (0.5, 1.9) | 1.1 (0.4, 3.9) | 0.2 |
| **Neutrophils** | 93 | 3 (1, 8) | 2 (1, 5) | 6 (2, 14) | 7 (4, 11) | **0.018** |
| **log10 viral load** | 97 | 4.54 (3.16, 5.35) | 5.07 (4.03, 5.55) | 3.58 (2.76, 5.10) | 3.48 (1.70, 4.33) | **<0.001** |
| **Sodium mmol/L** | 407 | 134.0 (130.0, 137.0) | 134.0 (130.0, 137.0) | 134.0 (130.0, 137.0) | 133.0 (130.0, 136.0) | >0.9 |
| **Potassium mmol/L** | 408 | 4.10 (3.60, 4.60) | 4.00 (3.60, 4.60) | 4.05 (3.70, 4.50) | 4.20 (3.60, 4.70) | 0.5 |
| **Opportunistic infections (OI)** |  |  |  |  |  |  |
| **Tuberculosis** | 428 | 312 (72.9%) | 155 (73.8%) | 83 (73.5%) | 74 (70.5%) | 0.8 |
| **Cryptococcus neoformans** | 428 | 1 (0.2%) | 1 (0.5%) | 0 (0.0%) | 0 (0.0%) | >0.9 |
| **Cytomegalovirus** | 428 | 1 (0.2%) | 0 (0.0%) | 1 (0.9%) | 0 (0.0%) | 0.5 |
| **Kaposi’s sarcoma** | 428 | 1 (0.2%) | 1 (0.5%) | 0 (0.0%) | 0 (0.0%) | >0.9 |
| **Other** | 428 | 114 (26.6%) | 50 (23.8%) | 31 (27.4%) | 33 (31.4%) | 0.3 |

**Clinical features of hypoadrenalism**

Hypoadrenalism was confirmed in 31 patients using the 30 minutes cortisol concentration of less than 500 nmol/L. ACTH stimulation resulted in stimulated cortisol 420 (338-473) nmol/L for AI vs 727 (640--859) nmol/L for non-AI patients *p*<0.001. There were no discernible differences in the demographic characteristics and symptoms of patients with hypoadrenalism versus those without. Among the clinical findings, only the median (IQR) diastolic BP was higher in the AI group, though not statistically significant at 71 (62-80) mmHg versus 69 (60-77) mmHg *p*=0.074. In respect of the clinical investigations, there were significant differences in the median (IQR) between the two groups with random cortisol 258 (210-370) nmol/L vs 486 (388-582) nmol/L *p*<0.001, and basal cortisol 300 (209-368) nmol/L versus 473 (368-580) *p*<0.001. The only significant differences between the PAI and SAI patients were the median (IQR) of 10 (7-10) versus 21(14-30) *p*=0.012 and ACTH levels 144(80-158) versus 23(12-23) *p*<0.001. There were no significant differences between the PAI and SAI in respect of demographics, history, clinical features, and investigations.

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| Table 2: Comparison of the clinical, investigations and mortality among AI vs Non-AI and PAI vs SAI groups | | | | | | |
| Variable | **Total AI, N = 31** | **PAI, N = 7** | **SAI, N = 24** | **p-value** | **No-AI, N = 400** | **p-value** |
| Age at enrolment, median (IQR) years | 36 (32, 46) | 40 (35, 45) | 36 (31, 45) | 0.6 | 36 (31, 42) | 0.3 |
| Female-gender, n (%) | 16 (51.6%) | 3 (42.9%) | 13 (54.2%) | 0.7 | 202 (50.8%) | >0.9 |
| Ethnicity, n (%) |  |  |  | 0.5 |  | 0.3 |
| Black African | 28 (90.3%) | 6 (85.7%) | 22 (91.7%) |  | 329 (82.9%) |  |
| Other | 3 (9.7%) | 1 (14.3%) | 2 (8.3%) |  | 68 (17.1%) |  |
| Duration of current illness, median (IQR) days | 14 (14, 30) | 10 (7, 12) | 21 (14, 30) | **0.012** | 14 (14, 21) | 0.4 |
| Random morning cortisol, median (IQR) nmol/L | 258 (210, 370) | 344 (267, 390) | 256 (193, 345) | 0.2 | 486 (388, 582) | **<0.001** |
| Basal cortisol, median (IQR) nnol/L | 300 (209, 368) | 315 (214, 327) | 300 (210, 378) | 0.6 | 473 (368, 580) | **<0.001** |
| Stimulated cortisol, median (IQR) nmo/L | 420 (338, 473) | 403 (363, 435) | 421 (326, 478) | 0.6 | 727 (640, 859) | **<0.001** |
| ACTH, median (IQR) pmol/L | 25 (14, 56) | 144 (80, 158) | 23 (12, 29) | **<0.001** | 32 (21, 51) | 0.5 |
| BP (systolic), median (IQR) mmHg | 120 (102, 128) | 120 (120, 124) | 115 (100, 128) | 0.5 | 110 (100, 124) | 0.4 |
| BP (diastolic), median (IQR) mmHg | 71 (62, 80) | 70 (70, 82) | 71 (60, 78) | 0.7 | 69 (60, 77) | 0.074 |
| Postural drop in blood pressure | 2 (6.5%) | 0 (0.0%) | 2 (8.3%) | >0.9 | 12 (3.2%) | 0.3 |
| Heart rate, median (IQR) bpm | 86 (78, 108) | 97 (88, 111) | 85 (77, 107) | 0.11 | 92 (81, 110) | 0.4 |
| Hypotension, n (%) | 1 (3.2%) | 0 (0.0%) | 1 (4.2%) | >0.9 | 29 (7.7%) | 0.7 |
| Weakness, n (%) | 25 (80.6%) | 4 (57.1%) | 21 (87.5%) | 0.11 | 318 (83.9%) | 0.6 |
| Tiredness, n (%) | 27 (87.1%) | 5 (71.4%) | 22 (91.7%) | 0.2 | 322 (84.7%) | >0.9 |
| Poor appetite, n (%) | 26 (83.9%) | 6 (85.7%) | 20 (83.3%) | >0.9 | 281 (74.5%) | 0.2 |
| Weight loss, n (%) | 25 (80.6%) | 7 (100.0%) | 18 (75.0%) | 0.3 | 338 (88.7%) | 0.2 |
| Increased pigmentation of the skin, n (%) | 11 (39.3%) | 3 (60.0%) | 8 (34.8%) | 0.4 | 169 (46.0%) | 0.5 |
| Nausea, n (%) | 18 (58.1%) | 5 (71.4%) | 13 (54.2%) | 0.7 | 191 (50.4%) | 0.4 |
| Vomiting, n (%) | 8 (25.8%) | 4 (57.1%) | 4 (16.7%) | **0.053** | 104 (27.5%) | 0.8 |
| Liking for salt, n (%) | 21 (67.7%) | 5 (71.4%) | 16 (66.7%) | >0.9 | 235 (62.5%) | 0.6 |
| Hypoglycaemia, n (%) | 31 (100.0%) | 0 | 0 |  | 9 (2.4%) | >0.9 |
| Loss of consciousness, n (%) | 30 (100.0%) | 0 | 0 |  | 5 (1.3%) | >0.9 |
| Diarrhea, n (%) | 9 (29.0%) | 1 (14.3%) | 8 (33.3%) | 0.6 | 153 (40.7%) | 0.2 |
| Dizziness, n (%) | 14 (46.7%) | 4 (66.7%) | 10 (41.7%) | 0.4 | 165 (43.8%) | 0.8 |
| Shock, n (%) | 31 (100.0%) | 0 | 0 | \_ | 5 (1.3%) | >0.9 |
| Anorexia, n (%) | 9 (29.0%) | 4 (57.1%) | 5 (20.8%) | 0.2 | 163 (43.1%) | 0.13 |
| Loss of axillary and pubic hair in females, n (%) | 3 (10.0%) | 1 (14.3%) | 2 (8.7%) | 0.6 | 65 (16.9%) | 0.6 |
| Presence of anaemia, n (%) | 15 (50.0%) | 4 (57.1%) | 11 (47.8%) | >0.9 | 208 (55.3%) | 0.6 |
| Presence of an opportunistic infection, n (%) | 31 (100.0%) | 7 (100.0%) | 24 (100.0%) |  | 393 (99.0%) | >0.9 |
| Viral load, median (IQR) (log10 Copies/mL) | 4.79 (4.07, 5.19) | 5.04 (5.04, 5.04) | 4.54 (3.61, 5.09) | >0.9 | 4.40 (3.16, 5.35) | 0.8 |
| Total CD4 count, median (IQR) | 39 (14, 50) | 46 (26, 61) | 35 (12, 50) | 0.3 | 31 (14, 60) | >0.9 |
| Sodium, median (IQR) mmol/L | 135.0 (132.5, 137.0) | 133.0 (131.5, 136.5) | 135.5 (133.8, 137.0) | 0.3 | 134.0 (130.0, 137.0) | 0.067 |
| Potassium, median (IQR) mmol/L | 3.90 (3.30, 4.35) | 3.60 (3.25, 3.85) | 3.95 (3.45, 4.52) | 0.3 | 4.10 (3.60, 4.60) | 0.12 |
| Haemoglobin, median (IQR) g/dL | 8.70 (7.80, 10.45) | 10.20 (7.85, 10.35) | 8.70 (7.85, 10.60) | 0.8 | 8.70 (7.40, 10.30) | 0.5 |
| White cell count, median (IQR) X109 | 5 (3, 7) | 4 (3, 5) | 5 (4, 11) | 0.3 | 5.4 (3.7, 8.1) | 0.7 |
| Lymphocyte count, median (IQR) X109 | 1 (1, 1) | 1 (1, 1) | 1 (0, 1) | 0.7 | 0.8 (0.3, 1.9) | 0.6 |
| Neutrophils, median (IQR) | 1.28 (0.95, 1.63) | 0.92 (0.92, 0.92) | 1.54 (1.02, 1.66) | 0.7 | 3 (1, 9) | 0.068 |
| Early Mortality, n (%) | 4 (28.6%) | 0 (0.0%) | 4 (36.4%) | 0.5 | 23 (16.3%) | 0.3 |
| Intermediate mortality, n (%) | 6 (40.0%) | 2 (50.0%) | 4 (36.4%) | >0.9 | 29 (19.3%) | 0.092 |
| Late mortality, n (%) | 7 (36.8%) | 2 (50.0%) | 5 (33.3%) | 0.6 | 37 (22.2%) | 0.2 |
| Tuberculosis, n (%) | 19 (61.3) | 4 (57.1%) | 15 (62.5%) | >0.9 | 294 (73.5%) | 0.14 |
| Cryptococcus neoformans, n (%) | 0 | 0 | 0 | >0.9 | 1 (0.3%) | > 0.9 |
| Cytomegalovirus, n (%) | 1 (3.2%) | 0 | 1 (4.2%) |  | 0 | 0.072 |
| Other opportunistic infections, n (%) | 13 (14.9%) | 3 (42.9%) | 10 (41.7%) | >0.9 | 101 (25.2%) | **0.042** |

**Morbidity and mortality outcomes:**

Overall, one year mortality was reported at (24.59%), with (22.25%) in the non-AI group, plus (54 %) in the AI group *p*=0.001. Most of the deaths 70/107 (65.42%) occurred early, followed by the late and intermediate at 19/107 (17.75%) and 18/107(16.82%), respectively. In contrast with non-AI group in which mortality occurred throughout the three stages, all the AI group deaths occurred in the last six months of the one-year follow-up. Although the PAI number was smaller, mortality in this group was 3/7 (57.14%) compared to the 14/31 (54.16%) in the SAI group.

**Table 3:** Causes of Mortality

|  |  |
| --- | --- |
| **System** | **N (%)** |
| **CNS:***Meningitis +, Cryptococcus, Encephalitis, trauma* | 10 (9.4%) |
| **TB/Pneumonia/COPD:** *TB / Pneumonia, MDR, Dissem, COPD, ILD* | 34 (32.1%) |
| **Liver failure:***LF, HBV* | 5 (4.7%) |
| **Kidney failure** | 10 (9.4%) |
| **GIT:** *Gastroenteritis, KS, Candida esophagitis, pancreatitis* | 9 (8.5%) |
| **Hematology:** *Sepsis, Cancer, Lymphomas* | 11 (10.4%) |
| **Natural causes** | 7 (6.6%) |
| **Unknown** | 25 (23.6%) |

**Linear regression and multivariate analysis.**

When we performed linear regression analyses, only random cortisol, basal cortisol and stimulated cortisol and Other opportunistic infections, were associated with AI. A 10 nmol/L increase in random cortisol was associated with an 18% increase in the odds of being diagnosed with AI, 1.18 (95%CI:1.13, 1.25). A 10 nmol/L increase in basal cortisol was associated with a 10% increase in the odds of being diagnosed with AI, 1.10 (95%CI:1.06, 1.15), while a 10 nmol/L increase in stimulated cortisol was associated with a 34% increase in the odds of being diagnosed with AI, 1.34 (95%CI:1.20, 1.59).

At multivariate analysis, after adjusting for lymphocyte count, both random morning cortisol and ACTH were independently associated with AI. A 10 nmol/L increase in random morning cortisol was associated with a 19.6% increase in the odds of being diagnosed with AI, 1.196 (95%CI:1.131, 1.265), while a 10 pmol/L increase in ACTH was associated with a 12.6% reduction in the odds of being diagnosed with AI, 0.874 (95%CI:0.786, 0.971) after adjusting for lymphocyte count.

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| --- | --- | --- | --- | --- | --- | --- |
| **Table** 4: Linear and Multivariate analysis | | | | | | |
| Variable | OR | 95% CI | p-value | Adj. OR | 95% CI | P-value |
| **SOCIO-DEMOGRAPHICS** |  |  |  |  |  |  |
| Age at enrolment, in years | 0.98 | 0.94, 1.02 | 0.3 |  |  |  |
| Female-gender | 1.03 | 0.50, 2.17 | >0.9 |  |  |  |
| Black-Ethnicity | 1.93 | 0.66, 8.23 | 0.3 |  |  |  |
| **HISTORY** |  |  |  |  |  |  |
| Duration of current illness (pre 10-day) | 0.98 | 0.95, 1.00 | 0.066 | 0.978 | 0.9954, 1.00009 | 0.0599 |
| Tiredness | 0.82 | 0.24, 2.20 | 0.7 |  |  |  |
| Poor appetite | 0.56 | 0.19, 1.39 | 0.3 |  |  |  |
| Weight loss | 1.89 | 0.67, 4.59 | 0.2 |  |  |  |
| Nausea | 0.73 | 0.34, 1.53 | 0.4 |  |  |  |
| Vomiting | 1.09 | 0.49, 2.67 | 0.8 |  |  |  |
| Liking for salt | 0.79 | 0.35, 1.69 | 0.6 |  |  |  |
| Diarrhoea | 1.68 | 0.77, 3.93 | 0.2 |  |  |  |
| Dizziness | 0.89 | 0.42, 1.90 | 0.8 |  |  |  |
| **CLINICAL** |  |  |  |  |  |  |
| Anorexia | 1.85 | 0.86, 4.34 | 0.13 |  |  |  |
| BP (systolic), mmHg | 1 | 0.98, 1.02 | 0.7 |  |  |  |
| BP (diastolic), mmHg | 0.98 | 0.96, 1.01 | 0.2 |  |  |  |
| Postural drop in blood pressure | 0.47 | 0.12, 3.14 | 0.3 |  |  |  |
| Heart rate, bpm | 1.01 | 0.99, 1.03 | 0.4 |  |  |  |
| Hypotension | 2.49 | 0.50, 45.2 | 0.4 |  |  |  |
| Weakness | 1.25 | 0.45, 3.00 | 0.6 |  |  |  |
| INVESTIGATIONS |  |  |  |  |  |  |
| Increased pigmentation of the skin | 1.32 | 0.61, 2.98 | 0.5 |  |  |  |
| Loss of axillary and pubic hair in female | 2.04 | 0.63, 9.12 | 0.3 |  |  |  |
| Random morning cortisol, nmol/L | **1.18** | **1.13, 1.25** | **<0.001** |  |  |  |
| Basal cortisol, nnol/L | **1.10** | **1.06, 1.15** | **<0.001** |  |  |  |
| Stimulated cortisol, nmo/L | **1.34** | **1.20, 1.59** | **<0.001** |  |  |  |
| ACTH, pmol/L | 1 | 0.99, 1.00 | 0.2 |  |  |  |
| Presence of anemia | 1.24 | 0.58, 2.62 | 0.6 |  |  |  |
| Viral load, log10 Copies/mL | 0.88 | 0.40, 1.74 | 0.7 |  |  |  |
| Total CD4 count, Cells/mL | 1 | 0.99, 1.02 | 0.8 |  |  |  |
| Sodium, mmol/L | 0.85 | 0.68, 1.09 | 0.2 |  |  |  |
| Potassium, mmol/L | 1 | 0.76, 1.76 | >0.9 |  |  |  |
| Haemoglobin, g/dL | 1 | 0.99, NA | 0.8 |  |  |  |
| White cell count, x109 | 0.99 | 0.98, 1.02 | 0.5 |  |  |  |
| Lymphocyte count, x109 | 0.82 | 0.54, 1.44 | 0.4 | 0.894 | 0.559, 1.43 | 0.6 |
| Neutrophils | 1.35 | 1.05, 2.52 | 0.2 |  |  |  |
| Early Mortality | 0.49 | 0.15, 1.90 | 0.3 |  |  |  |
| Intermediate mortality | 0.36 | 0.12, 1.15 | 0.071 |  |  |  |
| Late mortality | 0.49 | 0.18, 1.39 | 0.2 |  |  |  |
| Tuberculosis | 1.75 | 0.80, 3.69 | 0.15 | 1.731 | 0.77, 3.892 | 0.1835 |
| Other | **0.47** | **0.22, 1.01** | **0.047** |  |  |  |

The P-value for the Kaplan Meier is significant at 0.014 which means that there is a difference in mortality between AI and those without that diagnosis, especially in the late mortality.

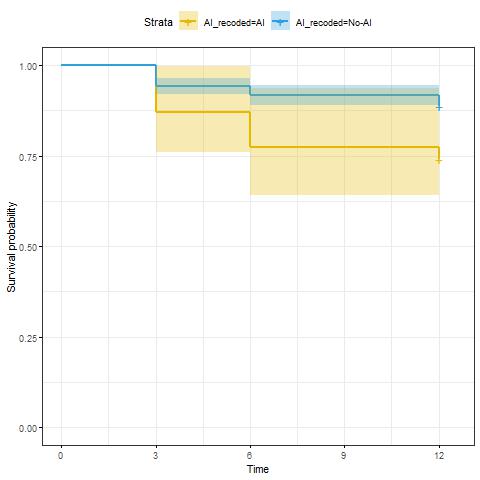


Fig 1: The Kaplan-Meier survival curve over time

Table X: multivariate model for mortality

|  |  |  |
| --- | --- | --- |
| Variable | Adj. HR (95% CI) | P-value |
| No Addison's | 0.437 (0.192, 0.9943) | 0.048495 |
| Gender | 0.821 (0.4618, 1.4596) | 0.493573 |
| Lymphocyte count | 0.998 (0.9381, 1.0612) | 0.935553 |
| Potassium in mmol.L | 0.888 (0.6919, 1.1392) | 0.341819 |
| Age at enrolment (years) | 1.021 (0.9897, 1.0532) | 0.186064 |

Patients without Addison’s were associated with a 56.3% reduction in the hazard of mortality compared to those with Addison’s 0.437 (95%CI: 0.192, 0.9943) P = 0.048, after adjusting for gender, age at enrolment, lymphocyte count, and potassium

**Discussion**

HIV is highly prevalent, and it has a high mortality, especially if patients are severely immunocompromised. We looked for patients with hypoadrenalism. The patients with hypoadrenalism 31/431, looked the same as the rest of the cohort in most respect except random, baseline and stimulated cortisol which were significantly lower in the hypoadrenal group. When comparing patients with hypoadrenalism, subdivided into primary 7/31 and secondary 24/31 hypoadrenalism they looked similar in respect of clinical features. When examining the etiology for primary and secondary hypoadrenalism we identified mainly tuberculosis at 73%, followed by a surprisingly low incidence of other OI’s such as cryptococcus (0.2%), CMV (0.2%), syphilis (0.2%) and HHV8 (Kaposi sarcoma). The overall median age at presentation was 36 years with the older age group associated with higher CD4 counts. Nausea, diarrhea and postural drop were predominant in the lowest tertile of CD4 count with significant p-values of *p*=0.013, p=0.020, and *p*=0.038, respectively. In respect of the investigations, higher white blood cells and neutrophil counts predominate in the higher CD4 tertile, while higher viral loads predominated in the middle CD4 count tertile with p-values at *p*<0.0001, *p*=0.018, and *p*<0.001, respectively.

Table 2: As expected, comparison of hypoadrenal patients with the rest of the cohort revealed statistically significant differences only in the random, baseline and stimulated cortisol levels. There were also differences in the median diastolic blood pressure and sodium levels which were higher in the hypoadrenal group, though not statistically significant. In contrast, the median neutrophil levels were higher in the rest of the cohort, though also not statistically significant. Neutrophil levels can be elevated by steroid-induced de-margination during inflammatory states, including HIV infection(17). We consider the lower neutrophil count in the AI group to be inappropriate and perhaps a marker of low cortisol state in advanced HIV which we consider to be a state of medical stress. Steroids can exert both pro and anti-inflammatory effect on the neutrophils depending on the inflammatory microenvironment (18). On the other hand, natural killer cell cytotoxicity (NKCC) lymphocyte dysfunction leading to increased infections has been associated with adrenal insufficiency(19), and increased mortality. In our study, the bivariate analysis a unit rise in lymphocytes was protective against AI, though not statistically significant.

Compared to the SAI group, the PAI group had a statistically significant shorter duration of illness at 10days versus 21days, higher median ACTH levels at 144 versus 23 and it also had a significantly higher incidence of vomiting at (57.1%) versus (16.7%), even though not statistically significant. The rest of the history, clinical signs and investigations of the PAI and the SAI groups were not significant. The surprising finding was the insignificant difference between the stimulated cortisol levels in both the PAI and SAI group.

The result of the Bivariate and multivariate analysis is counterintuitive, unless we assume that advanced HIV predicts higher cortisol output due to the metabolic and inflammatory stress, which may lead to adrenal cortisol depletion leading to AI.

**Morbidity & Mortality:**

The overall mortality at one year follow-up was statistically significant (*p*=0.0015) at 54.83% versus 22.25% for AI versus non-AI groups, respectively. Mortality occurred throughout the tertiles in non-AI groups. In contrast to the SAI mortality which occurred throughout the tertiles, the PAI group experienced intermediate to late mortality which was higher at 4/7 (57.14%) compared to the SAI group at 13/24 (54.16%), though not statistically significant. The cause of death varied from unknown to, natural, pulmonary and disseminated tuberculosis, meningitis, sepsis, organ failures such as kidney, liver and heart failure, and cancer.

**Strengths and Weaknesses:**

This is the largest prospective study of patients with advanced HIV in a draining area with a high background of tuberculosis. One weakness is the population selection bias with the black Africans making most of the participants due to the state hospital draining area being predominantly Black African.

**Conclusion:**

The largest study on hypoadrenalism in advanced HIV in Africa revealed overall mortality of 24.8%. The overall mortality in the AI group was 54.83% versus 22.25% for the non-AI groups, respectively. Although the PAI number was smaller, mortality in this group was alarming 57.14% compared to the 54.16% in the SAI group. Higher mortality in this group suggests that hypoadrenalism carries a higher risk of mortality in patients with advanced HIV and thus justifies screening for it in these patients.

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