Original Article

Occurrence of hypocortisolism in HIV patients: Is the picture changing?

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SUMMARY
Background: The occurrence of endocrine diseases in people who are infected with HIV is traditionally thought to occur in the setting of AIDS with opportunistic infections and malignancies. However, recent studies find the correlation between hypocortisolism and stage of HIV (CD4 count and WHO clinical stage) inconsistent.
Methods: This descriptive cross-sectional study included three hundred and fifty (350) consecutive patients with HIV infection. They were interviewed, and subsequently underwent laboratory evaluation for the detection of hypocortisolism. Blood samples for serum cortisol estimation were taken at baseline and at 30 minutes following the administration of 1μg of tetracosactrin (Synacthen). In addition, the patients had blood samples taken at 0 minutes (baseline) for CD4+ lymphocyte cell counts.
Results: At baseline, 108 (30.9%) participants had serum cortisol levels below 100 μg/L with a median value of 55.48 μg/L (11.36-99.96 μg/L), but only 57 (16.3%) study participants had stimulated serum cortisol levels below 180 μg/L with median of 118 μg/L (19.43-179.62). There was no significant difference in the occurrence of clinical features between participants with low and normal serum cortisol, nor WHO clinical stage, CD4 count and ART regimen. The occurrence of hypocortisolism was higher among participants who had been on ART for a longer period of time.
Conclusion: There is a high prevalence of hypocortisolism among HIV patients by biochemical testing, especially those who have been on ARVs for a longer duration. Hypocortisolism cannot be predicted based on the participants' WHO clinical stage of disease, CD4 cell count, or the treatment regimen.

Keywords: HIV, Adrenocortical insufficiency, CD4 cell count, Tetracosactrin

INTRODUCTION
The human immunodeficiency virus type-1 (HIV-1) infection and its sequelae, the acquired immune deficiency syndrome (AIDS) are major causes of morbidity and mortality worldwide, accounting for over 35 million deaths.[1] Endocrine diseases, including dyslipidemia, disorders of bone homeostasis, and dysfunction of the adrenal, gonadal, and thyroid axes were reported in HIV-infected persons early in the HIV epidemic prior to the availability of HAART.[2] The occurrence of endocrine diseases in people who are infected with HIV is traditionally thought to occur in the setting of AIDS with opportunistic infections and malignancies.[3, 4]

However, even in the setting of sustained treatment of HIV infection with highly active anti-retroviral therapy (HAART) and a fully suppressed HIV RNA, there is an associated heightened systemic inflammation and immune dysfunction, albeit at lower levels than without antiretroviral treatment.[5] This state of persistently heightened inflammation may account for certain endocrine and cardiovascular complications of HIV.[6]

Adrenal diseases are thought to be the commonest endocrine abnormality in HIV, with autopsy studies showing involvement of the adrenal glands in 40-90% of patients who have AIDS.[3,4,7]

A study done in Lagos, Nigeria among 44 newly diagnosed, treatment naïve persons with HIV infection reported a 34.8% prevalence of hypoadrenalism.[8]Meya*et al* found the prevalence of functional adrenal insufficiency in critically ill HIV patients in Makerere to be 19%.[9] A similar study done in South Africa involving 66 consecutive subjects diagnosed with HIV admitted to the medical wards of the Nelson Mandela Academic Hospital in a 3-month period revealed a prevalence of 27%.[10]

The occurrence of adrenal insufficiency among patients infected with HIV has been noted to occur more commonly among patients with advanced HIV infection with low CD4 counts and the presence of many opportunistic diseases.[3, 4] However, some studies have noted that the relationship between CD4 cell count and adrenal insufficiency has not been consistent and predictable, with the study in Lagos showing a poor correlation.[8]

The aim of this study was to evaluate the occurrence of hypocortisolism among HIV patients, and determine the relationship between hypocortisolism, the clinical stage of HIV, the CD4 count and the ART regimen of patients who were attending the HIV clinic at the Aminu Kano Teaching Hospital (AKTH), Kano.

METHODS
This study was descriptive cross-sectional study, conducted at AKTH, Kano. The study subjects included three hundred and fifty (350) consecutive patients with HIV infection presenting at the ART clinic of AKTH Kano. Recruited patients were adults (≥18 years), who were confirmed to have HIV infection according to the serial testing algorithm in accordance with the national guidelines. Participants were recruited from September 2015 to January 2016. All the recruited patients were interviewed for features suggestive of hypoadrenalism. They subsequently underwent laboratory evaluation for the detection of hypocortisolism. Patients presenting in Addisonian crisis, those who were on steroids, and other drugs that could interfere with adrenal function (e.g. ketoconazole, phenytoin, rifampicin) were excluded from the study.

Patients' clinical data were collected using an interviewer administered questionnaire. Blood samples for serum cortisol estimation were taken at baseline and at 30 minutes following the administration of 1μg of tetracosactrin (Synacthen). In addition, the patients had blood samples taken at 0 minutes (baseline) for CD4+ lymphocyte cell counts.The short Synacthen test was conducted in the morning between 8:00 AM and 9:00AM.

All the recruited participants had serum measurement both at baseline and 30 minutes after Synacthen administration. After explaining the procedure to the patient, 1μg of Synacthen was prepared by adding 250μg of tetracosactrin (Synacthen) (1mL) into 499 mL of normal saline and mixing the solution thoroughly. Each 2 mL of the diluted solution contained 1 μg tetracosactrin (Synacthen). The solution thus prepared was sealed after each usage and stored in a refrigerator at 4-8^{0}C.

After securing a venous access, the site was observed for the presence of hematoma or bleeding. One mL of normal saline was then injected to ensure patency of the vein, and 3 mL of blood was taken at 0 minutes, following which, 1 μg of Synacthen was injected intravenously. Another 3 mL of blood was drawn after 30 minutes. All the blood samples were kept on ice slabs and transported to the laboratory using ice packs for separation and storage of the serum at -20^{0}C until assayed. Estimation of serum cortisol was done after pooling of samples. The Calbiotech® Cortisol ELISA kit (Calbiotech Inc., Spring Valley, CA, USA) was used for the serum cortisol assay. Adrenal insufficiency was defined as less than 145 µg/L (400 nmol/L) using early morning basal serum cortisol and a 30-minute post-ACTH (stimulated) serum cortisol level of less than 180 µg/L (500 nmol/L).

All data were analyzed using Statistical Package for Social Sciences (SPSS), version 20.0, Chicago, IL USA. Participants' serum cortisol was presented as median with range while other quantitative variables like age and BMI were presented as means and standard deviation. The qualitative variables were presented as proportions and percentages. Statistical test of significance at 5% alpha level was done using the appropriate test statistic and were considered significant for *p* values < 0.05. The student t-test was used to compare the means of quantitative variables while the Mann-Whitney U test was used to compare the medians of the stimulated serum cortisol among participants with normal serum cortisol and those with hypoadrenalism. The χ^2 test was used as the test estimate for qualitative data. Logistic regression analysis was carried out to determine the predictors of hypocortisolism among the participants.

The Health Research and Ethical Committee of AKTH approved the study. Written informed Consent was obtained from each participant after demonstrating a satisfactory level of understanding regarding the nature and extent of their involvement and had willingly agreed to participate. Information and data derived were kept confidential and treated as such by the investigators.

RESULTS

The mean age of the study participants was 39.75 ± 9.22 years, with a mean of 40.0 ± 9.21years among those participants with a normal adrenal function and 38.49 ± 9.21 years among those with hypoadrenalism respectively (mean difference 1.51, 95% CI= -1.12 to 4.13, *p= 0.53*). Male gender constituted 49.1% (172) of the study participants while 50.9% (178) were female.

Serum cortisol

Analysis of the baseline serum cortisol showed that 108 (30.9%) of the study participants had serum cortisol levels below 100 μg/L with a median value of 55.48 μg/L (11.36-99.96 μg/L), in comparison to participants with a normal baseline serum cortisol, among whom the median serum cortisol was 150.26 μg/L (101-525 μg/L). (Median difference = 94.78, *p< 0.001,* Mann-Whitney U test)

Following the administration of 1 μg ACTH, 57 (16.3%) participants had measured serum cortisol values of less than 150 μg/L with a median value of 98 μg/L (19.43-149.62), while 293 (83.7%) had normal serum cortisol with a median serum cortisol of 221.69 μg/L (151.91-865.23). (Median difference = 123.69, *p<0.001,* Mann-Whitney U test)

Clinical features

The commonest observed symptom among participants with hypocortisolism was anorexia, which was observed among 13 (22.8%) participants with low stimulated serum cortisol, followed closely by weight loss, fever, lethargy and skin darkening. Abdominal pain occurred in only one participant with low stimulated serum cortisol (1.75%).

The most commonly documented examination finding among participants with low stimulated serum cortisol was pallor (22.8%). None of the participants with hypocortisolism had skin hyperpigmentation. Among the participants with normal stimulated serum cortisol levels however, 16.0% (47) had pallor, 2.0% (6) oral thrush while 1.4% (4) participants were noted to have hyperpigmentation of the skin.

There was no significant difference in the occurrence of various clinical features between participants with normal serum cortisol and those with hypocortisolism, except headache (*p= 0.02,* χ² test) which was commoner among those participants with hypoadrenalism (See Table 1).

WHO clinical stage of disease

Majority of the participants were in WHO clinical stage 1 of HIV infection, among whom 80.6% (170) had a normal serum cortisol while 19.4% (41) had hypocortisolism.The proportion of hypocortisolism relative to the total participants in each group shows that the greatest proportion of participants with hypocortisolism occurred within WHO clinical stage 1(19.4%) followed by those with stage 3 disease. The lowest proportion of participants with hypocortisolism occurred within participants with stage 4 disease (*p=0.27,* χ² test), as shown in Table 2.

Table 1 The distribution of clinical features of participants

Clinical feature	Hypoadrenalism (n = 57)	Normal (n = 273)	*p value**
SYMPTOMS			
Anorexia	13 (22.8%)	57 (19.5%)	0.56
Weight loss	10 (17.5%)	54 (18.4%)	0.87
Fever	9 (15.8%)	44 (15%)	0.88
Lethargy	9 (15.8%)	51 (14.6%)	0.77
Skin Hyperpigmentation	7 (12.3%)	30 (10.2%)	0.65
Body itching	6 (10.5%)	36 (12.3%)	0.71
Headache	6 (10.5%)	10 (3.4%)	0.02
Skin rashes	5 (8.7%)	29 (9.9%)	0.79
Abdominal pains	1 (1.75%)	9 (3%)	0.59
Diarhhoea	0	8 (2.7%)	0.21
Pallor	13 (22.8%)	47 (16%)	0.22
Oral thrush	2 (3.5%)	6 (2%)	0.49
Dyspigmentation	0	4 (1.4%)	0.38
Hepatomegaly	0	2 (0.7%)	0.53

*= χ² test

Duration from diagnosis of HIV

The highest proportion of the participants with low stimulated serum cortisol were found among participants who had been on treatment for a duration of between 6-10 years (20.4% [21 out of 103]) followed by those who had been on treatment for greater than 10 years (18.2% [4 out of 22]). Among the newly diagnosed (i.e. yet to commence HAART) participants, 13.6% (8 out of 59) had hypoadrenalism, while 14.5% (24 out of 166) of participants who had been on treatment for 1-5 years had hypoadrenalism (*p= 0.006,* χ² test).The participants that had adrenocortical insufficiency were noted to have had a longer duration from diagnosis compared to those that had a normal adrenal function (4.96 years versus 4.38 years, mean difference = -0.583, *p= 0.28*).

Serum CD4 count

The median current CD4 T lymphocyte count among participants with a low stimulated serum cortisol level was 466 cells/μL (46-1125 cells/μL), compared to CD4 cell counts of 372 cells/μL (14-1125 cells/μL), (Median difference = 94, *p= 0.10,* Mann-Whitney U test) in participants with a normal stimulated serum cortisol.

The participants with CD4 counts greater than 500 cells/µL had the highest prevalence of hypocortisolism, with 19.1% (22 out of 115) of the participants with CD4 counts greater than 500 cells/µL noted to have low stimulated cortisol. There was a negative correlation between the participants' current CD4 counts and measured serum cortisol, with correlations of -0.073 (p= 0.23) and -0.152 (p= 0.012) with the baseline and the stimulated serum cortisol concentrations respectively.

Table 2 Participants' clinical parameters and CD4 cell counts

Parameter	Category	Normo-adrenalism	Hypo-adrenalism	Proportion
WHO Clinical stage	Stage I	170	41	19.4%
	Stage II	69	9	11.5%
	Stage III	43	6	12.2%
	Stage IV	11	1	8.3%
BMI (Kg/m^2)	Underweight	43	7	14.0%
	Normal weight	145	25	14.7%
	Overweight	80	17	17.5%
	Obese	22	7	24.1%
CD4 Count (cells/ mm^3)	<200	55	7	11.3%
	200-500	145	28	16.2%
	>500	93	22	19.1%

ART regimen
The greatest proportion of the participants with low serum levels of stimulated cortisol were taking the AZT/3TC/NVP regimen (21.6% [24 out of 111]) followed by the TDF/3TC or FTC/EFV regimen (15.9% [21 out of 132]). Among participants who were yet to commence medications, 12.9% (8 out of 62) had hypocortisolism. A logistic regression analysis was conducted to predict the occurrence of hypocortisolism using duration of treatment, current CD4 count, WHO clinical stage, and treatment regimen as predictors. A test of the full model against a constant only model was not statistically significant, indicating that the predictors as a set did not reliably distinguish between the presence of hypocortisolism and normal serum levels (χ^2 = 17.99, p= 0.59, df = 20). Nagelkerke's R^2 of 0.12 indicated a weak relationship between prediction and grouping. Prediction success overall was 82.2% (99.1% for normal adrenal function and 2.1% for the occurrence of hypoadrenalism).

DISCUSSION
The highest proportion of study participants with hypoadrenalism as measured with stimulated serum cortisol measurement was observed among participants who had WHO clinical stage 1 disease, although it is worthy of note that this group also had the highest number of participants in the study. It was also noted in this study that the CD4 cell count was higher among participants who had hypocortisolism compared to those with normal adrenal function.

Similarly, majority of participants with hypocortisolism in this study as measured by stimulated serum cortisol had no distinguishing symptoms traditionally thought to be suggestive of adrenocortical insufficiency.

On the other hand, most of the patients with normal serum cortisol had features which may be seen in patients with adrenocortical insufficiency. This is consistent with findings by other authors who have noted a poor correlation between clinical features suggestive of hypoadrenalism in HIV patients with adrenal insufficiency.[8,9,11] In the study by Meya et al, symptoms of fatigue, anorexia, weight loss and vomiting all occurred with similar frequency among those who had adrenal insufficiency and those with normal cortisol function.[9]

Various studies done elsewhere have demonstrated a higher prevalence of hypoadrenalism in HIV patients with advanced disease (Stage 3 and 4), which may reflect opportunistic infections and malignancies that are more likely to occur in that group of patients due to declining immunological function.[11-13] Meya et al found that HIV stage 4 disease was associated with higher prevalence of adrenal insufficiency.[10] Even though the difference in median CD4 count between participants with hypoadrenalism and those with normal serum cortisol was not statistically significant, there was a significant inverse correlation between the CD4 count and the stimulated serum cortisol levels.

In this study, the highest proportion of patients with hypoadrenalism was on the AZT/3TC/NVP regimen. Due to the study design, a cause and effect relationship cannot be established for this drug regimen in relation to adrenocortical insufficiency. However, it is worthy of note that patients on this combination were more likely to have been on ARTs for a longer period than those on other combinations, and any effect of the drug may actually have been due to a longer duration of HIV infection and not due to the drug itself, as demonstrated in the inverse correlation between stimulated serum cortisol levels and duration of disease. It could be that the pattern of the occurrence of adrenal diseases in HIV patients is changing, and the aetiological factors responsible for hypoadrenalism may be shifting from opportunistic diseases to inflammatory mediators and metabolic disturbances that seem to persist even in the face of suppressed viral load,[4] especially in patients who have been on ARVs for a prolonged period of time (as was observed in this study). This changing picture may have ramifications for the current policy of early commencement of ARVs in all HIV infected patients irrespective of the CD4 counts.[14]

While numerous studies have shown morbidity and mortality benefits with early commencement of ARVs, [15, 16] there are lingering concerns about the long-term effects of the prolonged use of ARVs in this population. There is need therefore, for further study to clearly define the long-term effects of ARVs on the metabolic and endocrine outcomes of HIV patients, and establish whether there is indeed a changing picture in the occurrence of adrenal diseases in this group of patients.

This study was limited by our inability to evenly distribute the participants into the various WHO clinical stages by virtue of the fact that the sampling was a convenient one. Additionally, our inability to measure other adrenal hormones limited our inferences to adrenocortical function, and not hypoadrenalism.

CONCLUSION
There is a high prevalence of hypocortisolism among HIV patients by biochemical testing, especially those who have been on ARVs for a longer duration. The occurrence of hypocortisolism, however, cannot be predicted based on the participants' WHO clinical stage of disease, CD4 cell count, or the treatment regimen.

ACKNOWLEDGEMENT

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