

Assessment of Adreno-Cortical Function of Hiv/Aids Patients Using Salivary Cortisol.

^Abrahim Z.U, ^Byahaya I.A.

^{A,B}Department Of Chemical Pathology And Immunology, Aminu Kano Teaching Hospital, PMB 3452, Kano, Nigeria.

Abstract:

Background: Adrenal gland dysfunction has been reported as the most common and most serious endocrine disorder affecting HIV/AIDS patients. The non-specific presentation and associated high morbidity and mortality of adrenal dysfunction renders its assessment highly imperative. Salivary cortisol measurement is non-invasive, highly sensitive and specific for the diagnosis of adreno-cortical disorders.

Objective: To evaluate adreno-cortical function of HIV/AIDS patients using salivary cortisol.

Materials And Methods: A cross sectional study in which eighty HIV positive patients on Highly Active Anti-Retroviral Therapy (HAART) (group I), eighty HAART naïve HIV positive patients (group II) and eighty HIV negative controls (group III) were recruited. Baseline morning saliva samples were collected from all the subjects and analysed for cortisol using Elisa kits sourced from Salimetrics Europe Limited. A short synacthen test was conducted on subjects with low baseline salivary cortisol and a late night salivary cortisol (LNSC) measurement was performed on subjects with high baseline salivary cortisol.

Results: A statistically significant difference ($P < 0.005$) in the median salivary cortisol levels was observed between the study groups: group III > group I > group II (14.10 > 6.30 > 1.75).

The total prevalence of adreno-cortical dysfunction in all the study subjects was 13.8%. The HAART-treated patients had higher prevalence (17.5%) than the HAART naïve patients (10.0%). The prevalence of adreno-cortical hypofunction was higher than hyperfunction (8.1% and 5.6% respectively) among all the HIV positive patients.

Conclusion: HIV infection and HAART were associated with adreno-cortical dysfunction and salivary cortisol measurement is useful in diagnosis.

Keywords: adrenal cortex, AIDS, HAART, HIV, salivary cortisol.

I. Introduction

Adrenal dysfunction is the most common and most serious endocrine disorder affecting HIV/AIDS patients.^[1,2] However, a high index of suspicion is required to make the diagnosis because of its nonspecific presentation, for this reason it is often underdiagnosed due to limitations of the screening tests that are currently in use.^[3,4,5,6] Furthermore, there is paucity of local data on adrenal function in HIV patients in Nigeria, a country with a high burden of the disease. Salivary cortisol measurement offers greater advantage over serum cortisol because it is noninvasive, accurate, reliable, highly specific and sensitive for the evaluation of adrenocortical function.^[10]

II. Materials And Methods

This research work was a cross sectional study conducted between September, 2014 and February, 2015 in the Department of Chemical Pathology and Immunology, of a tertiary hospital in Nigeria. Two hundred and forty subjects were recruited for the study. They were made up of eighty type-1 HIV positive patients on HAART (group I), eighty HAART naïve type-1 HIV positive patients (group II) and eighty HIV negative apparently healthy volunteers as controls (group III). The HIV positive patients were attending the HIV clinic of the tertiary hospital, while the HIV sero-negative controls were apparently healthy individuals that presented to the blood donor clinic of the tertiary hospital for blood donation and mothers who were six weeks post-delivery that presented to the vaccination centre in the tertiary hospital for vaccination of their children.

Excluded from the study in order to reduce bias were subjects with morbid obesity, history of chronic alcohol use, history of chronic anxiety or depressive disorders, recent pregnancy, history of diabetes mellitus, history of prolonged steroid use, history of previous or current diagnosis of tuberculosis, history of previous or current diagnosis of fungal infection, history of oral disease or injury and history of previous diagnosis of hypercortisolism or hypocortisolism. The above information regarding the exclusion criteria were obtained from the study subjects during an interactive session prior to data collection and the information were subsequently confirmed from their case notes. Ethical approval to carry out this study was obtained from the Ethical Research Committee of the tertiary hospital. Informed and written consent were sought and obtained from each study

subject before the commencement of the study. Information on biodata were obtained from the subjects with the aid of structured pre-tested questionnaire. Saliva samples were collected after appropriate counselling at 8:00 am for the measurement of baseline morning salivary cortisol. Subjects were told to thoroughly rinse their mouth with water about ten minutes prior to sample collection. This is because presence of food particles in the mouth can interfere with the assay.^[11] Collection of sample was done by tilting the head of the subject forward, allowing saliva to pool on the floor of the mouth. Salimetrics oral swab was then placed under the tongue for about two to three minutes to ensure that it is soaked, after which it was removed and inserted into Salimetrics swab storage tube with the time and date of sample collection appropriately recorded.

Samples were then kept at 2^o - 6^oC after collection in order to avoid bacterial growth, and were later stored at -20^oC until analysis. On the day of analysis, samples were thawed and centrifuged at a relative centrifugal force (RCF) of 1500 X g, at 3000 revolution per minute using a centrifuge for 15 minutes. The saliva samples were then analysed for basal cortisol using Elisa kits sourced from Salimetrics Europe Limited. Each of the subject's basal salivary cortisol status was categorized into either low, high or normal salivary cortisol. Thereafter, subjects with low basal salivary cortisol results (< 2.6 nmol/l) were subjected to a standard dose (250 microgram) Synacthen stimulation test which adequately stimulates the adrenal glands,^[12] in order to confirm adreno-cortical hypofunction while those with high basal salivary cortisol results (> 43.0 nmol/l) were subjected to late night salivary cortisol (LNSC) testing in order to establish adreno-cortical hyperfunction. Loss of circadian rhythm and absence of late night nadir is the earliest biochemical abnormality seen in patients with hypercortisolism and this forms the basis for LNSC measurement.^[13]

The following outcome measures were used.^[11]

Salivary cortisol reference range: (8:00 am)

2.6 - 43 nmol/l

Late Night Salivary Cortisol (11:00 pm)

0.2 – 3.1 nmol/l (Normal individuals)

3.2 – 82nmol/l (Patients with Cushing's syndrome).

As for the subjects that required Synacthen stimulation test, saliva samples were collected for baseline salivary cortisol assay at 8:00 am, then Synacthen 250 microgram was given by intramuscular (IM) injection. Saliva samples were then collected for cortisol assay at 30 minutes and 60 minutes post IM Synacthen injection. Salivary cortisol concentration should increase from the baseline value to at least 20 nmol/l for a normal response. All data collected were entered into excel spread sheets. This was subsequently cleaned by filtering and sorting. The cleaned data was exported into Statistical Package for the Social Science (SPSS) version 16.0 for analysis. Mean and Standard Deviation (SD) were used to summarize quantitative data while proportions and percentages were used for qualitative data. Statistical analysis to compare qualitative variables was done using chi square test, while analysis of quantitative variables was done using student t-test. Nonparametric data was analyzed using nonparametric statistics (Kruskal-Wallis test). In all the analysis, 5% alpha level of significance which corresponds to 0.05 was considered as statistically significant.

III. Results

All recruited subjects completed the study with female preponderance over the males. The mean age of all study subjects was 35.9 ± 9.9 years. The HAART treated patients were slightly older with a higher mean age of 35.00 ± 9.0 years compared to the HAART naïve patients and the Controls who had mean age of 34.4 ± 10.1 years and 33.0 ± 9.3 years respectively (Table I). The age group with the highest number of subjects in the three study groups was between 25-29 years. (figure1). There was a statistically significant difference in the median basal salivary cortisol of the three groups (p < 0.005), with a median basal salivary cortisol of 6.30 nmol/L for group I, 1.75 nmol/L for group II and 14.10 nmol/L for group III respectively (Table II).

Table III shows the distribution of baseline SC status of the study subjects. There were more subjects with abnormal levels of basal SC in group I than group II. All subjects in group III had normal basal cortisol levels (figure 2). Table III shows response of SC to short synacthen test. The baseline SC level and 30 minute post synacthen test SC increments were lower in group I than in group II but the difference was not statistically significant. However, the difference in SC response to synacthen at 60 minutes was statistically significant between the two groups (p < 0.005). Table IV shows the mean LNSC concentrations of study subjects with high basal SC. Group I subjects had higher mean LNSC concentrations than group II subjects. However, the difference in mean concentration of LNSC among the two groups was not statistically significant Table V shows that the prevalence of adreno-cortical hypofunction was higher among group I subjects (11.3%) than group II subjects (5.0%). However, the overall prevalence of adreno-cortical hypofunction was 8.1%. The prevalence of adreno-cortical hyperfunction was also higher among group I subjects (6.3%) than group II subjects (5.0%), while the combined prevalence of adreno-cortical hyperfunction observed in all study subjects

was 5.6%. (Table VI). Adreno-cortical hypofunction was found to be the most prevalent adrenocortical dysfunction (prevalence of 8.1%) among the study subjects and the prevalence is higher among group I subjects (11.3%) than group II subjects (5.0%). The prevalence of adreno-cortical hyperfunction was also observed to be higher in group I (6.3%) than in group II (5.0%). (Figure 2).

IV. Discussion

This work was conducted to determine the magnitude of adrenocortical dysfunction in HIV/AIDS patients. This has become necessary because adrenal gland dysfunction was reported to be the most common and most serious endocrine disorder affecting HIV/AIDS patients,^[2] and the disorder has an associated high morbidity and mortality.^[1] A high index of suspicion is required to make the diagnosis as a result of its non-specific presentation in most cases. Findings from this study will therefore contribute positively to future interventional studies and improve patient outcomes.

Salivary cortisol measurement was used instead of the conventional serum cortisol because it is simple, non-invasive, reproducible, highly sensitive (92% - 100%) and specific (84.9% - 100%).^[15] Salivary cortisol levels reflect the free and thus the active form of cortisol which can be reliably used in patients with increased or decreased plasma binding proteins e.g during critical illness, while serum cortisol levels reflect total cortisol (free and bound forms) levels.^[16] Though 24-hour urinary free cortisol (UFC) estimation also reflects the active form of cortisol, the required 24-hour urine collection poses difficult challenge to the patients due to incomplete urine collection which usually results in under estimation of cortisol. UFC estimation is also faced with non-standardized laboratory assay methods with consequent falsely low results, especially in patients with renal impairment. Studies have consistently reported that salivary cortisol follows the diurnal rhythm of serum cortisol,^[20,21,22,] and also highly significant correlations between serum and salivary cortisol levels ($p < 0.0001$) indicating that salivary cortisol can reliably replace serum cortisol.^[11,23,24,25] A study done to compare serum and salivary cortisol in the diagnosis of adrenal insufficiency reached a conclusion that salivary cortisol may be a better laboratory indicator of cortisol levels than serum total cortisol. The sex distribution of the study subjects showed a female preponderance of 59.6%. This suggests that HIV infection was commoner among females than males in the area of study. This is in keeping with the findings of studies conducted in South-east Nigeria and North-central Nigeria in which female preponderance of 68.9% and 51.5% were reported respectively.^[27,28] Similarly, other studies conducted outside Nigeria also reported female preponderance.^[29,30,31,32,33] However, a study conducted by Iliyasu and colleagues reported male preponderance of 54.6% among HIV infected individuals. This study has shown that HAART-treated HIV patients were slightly older and had a higher mean age compared to the HAART-naïve group. This may be due to the effect of HAART in improving the life expectancy of these patients, thus enabling them to live longer. The age group with the highest number of subjects in both the HAART-treated group and HAART-naïve group was 25-29 years. This suggests that HIV infection was highest among young individuals in the area of study. This observation agrees with the findings of United Nation's progress report on Nigeria,^[29] as well as the reports of other studies conducted globally.^[28,29,31,32,]

The median basal salivary cortisol of HAART-treated HIV positive patients was lower than that of the HAART-naïve group. The difference in median basal salivary cortisol between the two groups was statistically significant ($P = 0.01$). The observed difference may be due to the effect of HAART on the adrenal gland as well as the direct effect of the virus in causing destruction of the gland.^[1] The control group had higher median basal salivary cortisol than both HAART-treated and HAART-naïve groups. This observation agrees with the findings of a study conducted in Israel by Perlman and colleagues, who reported a higher mean basal salivary cortisol levels for the control group compared to patients with hypoadrenalism. All the subjects with low basal SC in both HAART-treated and HAART-naïve groups responded inadequately to the short Synacthen test at 30 minutes and 60 minutes post Synacthen injection. However, the response at 60 minutes was significantly higher among the HAART-naïve group than HAART-treated group ($P = 0.001$). This agrees with the findings of Contreras and co-researchers who observed blunted salivary cortisol response to standard dose Synacthen test in all patients with adrenal insufficiency.^[14] Cardoso and co-workers also reported that 8 out of 21 HIV positive patients responded inadequately to low dose Synacthen test.^[38] Odeniyi and co-workers in Lagos Nigeria, also reported that the cortisol response to Synacthen test was significantly lower in persons with HIV than healthy subjects.^[39]

The mean late night salivary cortisol (LNSC) concentration was high in all subjects with high basal SC in both groups I and II. This finding is similar to that observed by Raff and colleagues in Wisconsin where they reported a mean LNSC of 24.0 ± 4.5 nmol/L for patients with proven Cushing's syndrome as compared to normal subjects who had mean LNSC of 1.2 ± 0.1 nmol/L.^[40] High LNSC concentration which confirms loss of diurnal rhythm in cortisol secretion is the earliest manifestation seen in patients with hypercortisolism.^[13] HIV infection may result in hypercortisolism via the effect of cytokines on the hypothalamo-pituitary-adrenal (HPA) axis.^[1] It is postulated that HIV infection triggers macrophages to secrete interleukins e.g (IL-1) and tumour

necrosis factor (TNF) which act as adrenal stimulators.^[1] HIV infection of the brain stimulates the secretion of Interleukin-1 which enhances the release of corticotrophin releasing hormone (CRH) from the hypothalamus to the circulation thereby resulting in increased secretion of adrenocorticotrophic hormone (ACTH) which subsequently leads to increased cortisol secretion. Findings of several studies, depending on the study design or study population, have reported varying prevalence rates of adrenocortical dysfunction among HIV infected patients between 2.8% - 74.0%.

Findings from this study revealed that adreno-cortical hypofunction (hypocortisolism) was the most prevalent form of adrenal dysfunction among the study subjects with a prevalence of 8.1%. The prevalence was higher among the HAART treated HIV patients (11.3%) than the HAART naïve HIV patients (5.0%). Adreno-cortical hyperfunction (hypercortisolism) was also more prevalent among HAART treated HIV positive patients (6.3%) than the HAART naïve HIV positive patients (5.0%). The total prevalence of adreno-cortical hyperfunction among all the study subjects was 5.6%. The prevalence of adreno-cortical dysfunction (hypofunction and hyperfunction combined) was higher among HAART treated group (17.5%) than the HAART naïve group (10.0%). This finding may be due to the effect of HAART on the adrenal gland in addition to the direct effect of the virus on the gland. Overall, the total prevalence of an adreno-cortical dysfunction among all study subjects was found to be 13.8%.

Meyaet *al* in Uganda reported a prevalence of adrenal insufficiency of 19%.^[43] This could be due to the fact that both Uganda and Nigeria are resource limited settings that share similar socio-demographic patterns. The slightly higher prevalence observed in the Uganda study may be because they conducted their study among critically ill HIV patients that were admitted into the medical emergency unit while this study was conducted among stable HIV patients attending routine follow-up clinic. Gonzalez and colleagues in Mexico, reported a 21.2% prevalence of adrenal insufficiency (AI) among HIV patients. Their study further classified the participants into AIDS and HIV group. The prevalence of AI was higher among the AIDS group than the HIV group (26.4% and 9.4% respectively).^[48] Ekpebegh and colleagues in South Africa also reported a 27% prevalence of hypoadrenalism among patients with HIV infection.^[49] There are other studies that have reported similar prevalence of an adreno-cortical dysfunction among HIV patients^[50,51,52] However, very high prevalence of AI (74%) was reported by Shashidar and his colleague among HIV patients.^[47] The higher prevalence observed may be because they used critically ill HIV patients and they also used the lower dose of Synacthen (1 µg) which might not have adequately stimulated the adrenal gland thereby resulting in over-diagnosis of adrenal insufficiency in the study subjects.^[47]

V. Conclusion

This study has established that adreno-cortical dysfunction is common among both HAART treated and HAART naïve HIV positive patients. Adreno-cortical hypofunction was the commonest form of adrenal dysfunction among the two groups of HIV positive subjects, with higher prevalence in the HAART-treated group. Adreno-cortical hyperfunction was also commoner among HIV HAART-treated patients than the HAART naïve group.

Based On The High Prevalence Of Adreno-Cortical Dysfunction Found In This Study, The Following Are Recommended:

1. Baseline morning salivary cortisol measurement should be routinely conducted in all newly diagnosed HIV positive patients in order detect and treat dysfunction of the adrenal cortex early in the disease.
2. All HAART eligible patients should have salivary cortisol measurement done with a view to establishing a baseline prior to commencement of HAART.
3. The treatment of patients with adrenal dysfunction should be offered immediately because of the associated high morbidity and mortality, since adrenal insufficiency is the commonest and most serious endocrine complication that occurs in persons with HIV infection.
4. Clinicians especially Endocrinologists and Chemical Pathologist should be encouraged to conduct more studies with a view to establishing guidelines for treatment of HIV patients with adrenal dysfunction and also to investigate outcomes in patients treated for adrenal dysfunction.

References

- [1]. N.Jain, M. Mittal, H. Dandu, P.S. Verma, M. Gutch, K.M. Tripathi, et al, An observational study of endocrine disorders in HIV infected patients from North India, *Journal of HIV and Human Reproduction*, 1(1), 2013, 20-24.
- [2]. M.S. Eledrisi, A.C. Verghese, Adrenal insufficiency in HIV infection: a review and recommendations, *American Journal of Medical Sciences*, 32(12), 2001, 137-44.
- [3]. A.D. Papanicolaou, N. Mullen, L. Kyrou, L.K. Nieman, Night time salivary cortisol: A useful test for the diagnosis of Cushing's syndrome, *Journal of Clinical Endocrinology and Metabolism*, 87(10), 2002, 4515-4521.
- [4]. T. Carol, H. Raff, J.W. Findling, Late-night salivary cortisol measurement in the diagnosis of Cushing's syndrome, *Nature Clinical Practice Endocrinology and Metabolism*, 4(6), 2008, 344-50.

- [5]. S. Sakihara, K. Kaqeyama, Y. Oki, M. Doi, Y. Iwasaki, S. Takayasu, et al, Evaluation of plasma, salivary and urinary cortisol levels for the diagnosis of Cushing's syndrome, *Endocrinology Journal*, 57(4), 2010, 331-7.
- [6]. A.O. Ifedayo, A.F. Olufemi, Urine free cortisol in the diagnosis of Cushing's syndrome: How useful?, *Nigerian Journal of Clinical Practice*, 16(3), 2013, 269-272.
- [7]. Federal Republic of Nigeria, Global AIDS Response Country Progress Report (GARPR), 2012, National Agency for The Control of AIDS (NACA).
- [8]. Federal Republic of Nigeria, National Agency for Control of AIDS (NACA), Global AIDS Response Country Progress Report (GARPR), 2014.
- [9]. Federal Republic of Nigeria, National Agency for Control of AIDS (NACA), Global AIDS Response Country Progress Report (GARPR), 2015.
- [10]. H. Raff, Utility of salivary cortisol measurements in Cushing's syndrome and adrenal insufficiency, *The Journal of Clinical Endocrinology & Metabolism*, 94(10), 2009, 3647-3655.
- [11]. Salimetrics Europe Limited, Suffolk, CB8 7SY, UK, High Sensitivity Salivary Cortisol Enzyme immunoassay package insert, February 2010. Available at www.salimetrics.com.
- [12]. N. Nicola, N. Lynnette, Adrenal Insufficiency: etiology, diagnosis and treatment, *Current Opinion in Endocrinology, Diabetes & Obesity*, 17(13), 2010, 217-223.
- [13]. L.K. Nieman, B.M.K. Biller, V.M. Montori, The Diagnosis of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline, *The Journal of Clinical Endocrinology & Metabolism*, 93(5), 2008, 1526-1540.
- [14]. L.N. Contreras, A.L. Arreger, G.G. Persi, N.S. Gonzalez, E.M. Cardoso. A new less-invasive and more informative low dose ACTH test: salivary steroids in response to intramuscular corticotrophin. *Clinical Endocrinology (Oxford)*, 2004, 675-82.
- [15]. D. Erickson, S. Pitale, A.S. Dejong, Adrenal disorders, in P.M. Camacho, H. Gharib (Eds.), *Evidence based Endocrinology*, 2nd ed, (Chicago:Lippincott Williams & Wilkins, 2007) 66-86.
- [16]. R.J. Peter, F.R. Walker, R.D. Fahmy, R. Hall, Salivary cortisol assays for assessing Pituitary adrenal reserve. *Clinical Endocrinology*, 2008. Available from DOI: 10.1111/j.1365.1982.tb1631.x
- [17]. A.D. Papanicolaou, N. Mullen, L. Kyrou, L.K. Nieman, Night time salivary cortisol: A useful test for the diagnosis of Cushing's syndrome, *Journal of Clinical Endocrinology and Metabolism*, 87(10), 2002, 4515-4521.
- [18]. T. Carrol, H. Raff, J.W. Findling, Late-night salivary cortisol measurement in the diagnosis of Cushing's syndrome, *Nature Clinical Practice Endocrinology & Metabolism*, 4(6), 2008, 344-50.
- [19]. S. Sakihara, K. Kaqeyama, Y. Oki, M. Doi, Y. Iwasaki, S. Takayasu S, et al, Evaluation of plasma, salivary and urinary cortisol levels for the diagnosis of Cushing's syndrome. *Endocrinology Journal*, 57(4), 2010, 331-7.
- [20]. Y-M.R.M. Estrada, P.R. Orlander, Salivary cortisol can replace free serum cortisol measurement in patients with septic shock. *Chest*, 140(5), 2011, 1216-22. Available from doi: 10.1378/chest.11-0448.Epub 2011 Aug 4.
- [21]. J.I. Warrick, D. Goce, R. Anthony, Measurement of salivary cortisol in 2012—laboratory techniques and clinical indications, *Clinical Endocrinology*, 77(5), 2012, 645-651.
- [22]. G.L. Adriana, H.H. Amir, *Diseases of the Adrenal Gland*, Cleveland Clinic, 2010, Center for Continuing Education.
- [23]. M. Trilk, J. Flitsch, D.K. Ludecke, R. Junq, S. Petersenn, Salivary cortisol measurement—a reliable method for the diagnosis of Cushing's syndrome, *Experimental Clinical Endocrinology & Diabetes*, 113(4), 2005, 225-30.
- [24]. K. Lovas, E.S. Husebye, Salivary cortisol in adrenal diseases. *TidsskrNorLaegeforen*, 127(6), 2007, 730-2.
- [25]. P. Restituto, J.C. Galofre, M.Y. Gil, C. Milqueta, S. Santos, J.I. Monreal, et al, Advantages of salivary cortisol measurements in the diagnosis of glucocorticoid related disorders, *Clinical Biochemistry*, 41(9)2008, 688-92.
- [26]. R.C. V. Mello, E.F. Sad, B.C. Andrade, S.P.F. Neves, S.M.E. Santos, S.M.M Sarquis, et al, Serum and salivary cortisol in the diagnosis of adrenal insufficiency and as a predictor of the outcome in patients with severe sepsis. *Arquivos Brasileiros de Endocrinologia & Metabologia*. [Internet]. 2011 October; 55 (7). Available from <http://dx.doi.org/10.1590/S0004-27302011000700004>.
- [27]. C.M. Nwozor, J. Nwankwo, CD4 Cell Count of HIV-Positive Patients in Awka, South East Nigeria, *Greener Journal of Epidemiology and Public Health*, 1(2), 2013, 10-15.
- [28]. J.G. Laah, E. Ayiwulu, Socio-Demographic Characteristics of Patients Diagnosed with HIV/AIDS in Nasarawa Eggon, *Asian Journal of Medical Sciences*, 2(3), 2010, 114-120.
- [29]. Report of the United Nations General Assembly Special Session. Country progress report: Nigeria, 2010.
- [30]. N.F. Nguemai'm, J. Mbuagbaw, T. Nkoa, G. Alemnji, G. Te'to, T. Fanhi, et al, Serum lipid profile in highly active antiretroviral therapy-naïve HIV-infected patients in Cameroon: a case-control study, *HIV Medicine*, 11, 2010, 353-359.
- [31]. A.E. Pettifor, H.V. Rees, I. Kleinschmidt, Young people's sexual health in South Africa: HIV prevalence and sexual behaviors from a nationally representative household survey. *AIDS* 19, 2005, 1525-1534.
- [32]. Center for Disease Control. Fact sheet: Estimates of New HIV Infections in the United States, 2007-2010. [homepage on the Internet]. [updated 2012 Dec 19; cited 2013 Jan 9]. Available from <http://www.cdc.gov/hiv/topics/surveillance/incidence.htm>
- [33]. L. Annison, A. Dompok, Y. Adu-Sarkodie, The Immunological Response of HIV-Positive Patients Initiating HAART at the KomfoAnokye Teaching Hospital, Kumasi, Ghana. *Ghana Medical Journal*, 47(4), 2013; 47(4),164-170.
- [34]. Z. Iliyasu, J.T. Arotiba, M.Babashani, Socio-demographic characteristics and risk factors among HIV/AIDS patients in Kano, Northern Nigeria, *Nigeria Journal Medicine*, 14(4), 2005, 459-60.
- [35]. U. Sinha, N. Sengupta, P. Mukhopadhyay, K.S. Roy, Human Immunodeficiency Virus Endocrinopathy, *Indian Journal Endocrinology and Metabolism*, 15(4), 2011, 251-260.
- [36]. Federal Ministry of Health Abuja-Nigeria. National guidelines for HIV and AIDS treatment and care in adolescents and adults. 2010.
- [37]. Y.M. Perlman, K. Tordjman, Y. Greenman, R. Limor, G. Shenkerman, E. Osher, et al, Low-dose ACTH (1 microg) salivary test: a potential alternative to the classical blood test, *Clinical Endocrinology (Oxford)*, 64(2), 2006, 215-8.
- [38]. E. Cardoso, G. Persi, N. González, O. Tumilasci, A. Arreger, M. Burgos, et al, Assessment of adrenal function by measurement of salivary steroids in response to corticotrophin in patients infected with human immunodeficiency virus, *Steroids*, 72(4), 2007, 328-34.
- [39]. I.A. Odeniyi, O.A. Fasanmade, M.O. Ajala, A.E. Ohwovoriole, Adrenocortical Function in Nigerians With Human Immunodeficiency Virus Infection, *Ghana Medical Journal*, 47(4), 2013, 171-177.
- [40]. R. Hershel, L.R. Jonathan, W.F. James, Late-Night Salivary Cortisol as a Screening Test for Cushing's Syndrome, *Journal of Clinical Endocrinology and Metabolism*, J2013; [Internet]. Available from <http://dx.doi.org/10.1210/jcem.83.8.4936>
- [41]. B.A. Girei, S.B. Fatima, Endocrine manifestations of HIV infection, in K.S. Saxena (Ed), *Current Perspectives in HIV Infection*, (Intech; 2013) 243-259. Available from <http://dx.doi.org/10.5772/52684>.

- [42]. C.E. Klatt, Pathology of AIDS, Version 23, 17(3), 2012, 205-209.
- [43]. B.D. Meya, E. Katabira, M. Otim, A. Ronald, R. Colebunders, D. Njama, et al. Functional adrenal insufficiency among critically ill patients with Human immunodeficiency virus in a resource-limited setting, African journal of Health Sciences, 7(2), 2007, 101-107.
- [44]. V. Prasanthai, S. Sunthornyothin, P. Phowthongkum, C. Suankratay, Prevalance of Adrenal insufficiency in critically ill patients with AIDS, Journal of Medical Association of Thailand, 90(9), 2007, 1764-1774.
- [45]. I.A. Odeniyi, O. Fasamande, M. Ajala, A.E. Ohwovoriole, Adrenocortical function in Nigerian Persons with Human Immunodeficiency Virus (HIV) infection. Abstracts-2011 American Association of Clinical Endocrinologist, 2011.
- [46]. C. Julios, M. Jose, M. Eduardo, I. Sofia, Serum cortisol in HIV-infected patients with and without highly active anti-retroviral therapy, AIDS, 17(1), 2003, 123-126.
- [47]. K.P. Shashidhar, V.P. Shashikala, Low dose adrenocorticotrophic hormone test and adrenal insufficiency in critically ill acquired immunodeficiency syndrome patients. Indian Journal of Endocrinology and Metabolism, 16(3), 2012, 389-394.
- [48]. G.J.G. González, N.E. Garza-Hernández, R.A. Garza-Morán, M.I.M. Rivera, J. Montes-Villarreal, J. Valenzuela-Rendón, et al, Prevalence of abnormal adrenocortical function in human immunodeficiency virus infection by low-dose cosyntropin test. International Journal of STD & AIDS, 12(12), 2001, 804-10.
- [49]. C.O. Ekpebeghe, A.O. Ogbera, B. Longo-Mbenza, E. Blanco-Blanco, A. Awotedu, P. Oluboyo, Basal Cortisol Levels and Correlates of Hypoadrenalism in Patients with Human Immunodeficiency Virus Infection, Medical Principles and Practice, 20(6), 2011. Available at DOI:10.1159/000330022.
- [50]. P.I. Ezeugwunne, C.C. Onyenekwe, E.J. Ahaneke, M. Ifeanyichukwu, C.S. Meludu, W.O. Onwurah, et al, Serum hormonal levels in HIV/AIDS infected males on anti retroviral therapy (ART) in Nnewi, Nigeria, African Journals on line, 6(4), 2012. [Internet]. Available from <http://dx.doi.org/10.4314/ijbcs.v6i4.1>.
- [51]. J. Lo, S.K. Geenspoon, Adrenal function in HIV infection, Current Opinion Endocrinology Diabetes and obesity, 17(3), 2010, 205-209.
- [52]. R. Gobind, HIV and Anaesthesia. University of Kwazulu-Natal.

Table I: Sex and Age distribution of the study subjects.

Variables	Variables	Group I	Group II	Group III	Total
Sex	Male (N %)	43 53.8	27 33.6	41 51.3	40.4
	Female (N %)	37 46.2	53 66.4	39 48.7	59.6
Age In Years	Mean ± Sd	35.00 ± 9.0	34.4 ± 10.1	33.0 ± 9.3	35.9 ± 9.9

Table II: Basal salivary cortisol (Median, minimum and maximum values) of the study subjects

Group	Summarizing Indices	Baseline Cortisol (Nmol/L)
Group 1	Median	6.3
	Minimum Value	0.9
	Maximum Value	78.7
	Range	77.8
Group 2	Median	1.75
	Minimum Value	0.1
	Maximum Value	72.3
	Range	72.2
Group 3	Median	14.1
	Minimum Value	3.3
	Maximum Value	38.2
	Range	34.9
P-Value		<0.005

Table III: Response of Salivary Cortisol (mean ± SD) to Short Synacthen Test in HIV patients with low baseline SC by group.

Category	0 min Cortisol (nmol/L)	30 min Cortisol (nmol/L)	60 min Cortisol (nmol/L)
GROUP I (n = 9)	1.81 ± 0.53	8.16 ± 4.31	7.22 ± 2.79
GROUP II (n = 4)	1.95 ± 0.33	12.65 ± 4.25	14.68 ± 1.85
p- value	<0.05	0.11	<0.005

Table IV: Mean Late Night Salivary Cortisol (LNCS) concentrations of HIV patients With High baseline SC by group.

Category	1 st measurement	2 nd measurement	Mean LNCS
GROUP I (n = 5)	26.42 ± 4.46	24.32 ± 4.48	25.74 ± 4.47
GROUP II (n = 5)	16.30 ± 4.01	18.00 ± 6.00	17.30 ± 5.01

Table V: Prevalence Of An Adreno-Cortical Hypofunction Among Study Subjects.

Category	Low Cortisol N (%)	Baseline	Suboptimal Response To Synacthen Test N (%)	Prevalence Of Hypofunction (%)
Group I	9 (11.3)		9 (11.3)	11.3
Group II	4 (5.0)		4 (5.0)	5.0
Total	13 (8.1)		13 (8.1)	8.1

Table VI: Prevalence of an adreno-cortical hyperfunction among study subjects

Category	High Cortisol n (%)	Baseline	High LNSC n (%)	Prevalence of hyperfunction (%)
GROUP I	5 (6.3)		5 (6.3)	6.3
GROUP II	4 (5.0)		4 (5.0)	5.0
TOTAL	9(5.6)		9(5.6)	5.6