ADRENOCORTICAL FUNCTION IN NIGERIANS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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SUMMARY

Objective: This study sets out to determine the prevalence of adrenocortical insufficiency in persons with HIV infection by determining the response to low-dose (1µg) ACTH stimulation

Design: An experimental study involving people with HIV infection and healthy people.

Setting: The study group and the controls were recruited from the Lagos University Teaching Hospital (LUTH).

Participants: forty-three newly diagnosed and treatment naïve persons with HIV (23 males and 20 females) and 70 (35 males and 35 females) HIV negative subjects completed the study.

Intervention: One µg Synacthen was given intravenously to stimulate the adrenal glands.

Main outcome measures: Blood was collected for basal cortisol levels and 30 minutes after the injection of ACTH. Cortisol was assayed using ELISA.

Results: The mean basal cortisol was 154.9 ± 27.2 nmol/L and 239.9 ± 31.6 nmol/L (p<0.001); while the 30-minute post ACTH test cortisol level was 354.8 ± 19.9 nmol/L and 870.9 ± 163.5 nmol/L (p<0.001) and the increment was 100.0 ± 17.2 nmol/L and 588.8 ± 143.4 nmol/L (p<0.001) in HIV and healthy subject group respectively. Using the diagnostic criteria derived for the diagnosis of adrenocortical insufficiency in this study (30 minute cortisol level <380.2nmol/L and increment from basal to stimulated cortisol level <158.5nmol/L); fifteen (34.8%) persons with HIV had adrenal insufficiency.

Conclusion: Adrenocortical insufficiency is common in persons with HIV infection, occurring in about 34.8% of patients studied. Clinically evident adrenocortical insufficiency is uncommon in persons with HIV.

Keywords: Cortisol; Adrenocorticotropic hormone (ACTH); Human Immunodeficiency Virus (HIV).

INTRODUCTION

Adrenal gland involvement has been documented in as many as two thirds of patients with human Immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS) at post-mortem examination.[1] However, adrenal insufficiency is seldom diagnosed in clinical practice because symptoms do not appear until more than 80% of the gland has been destroyed[1].

The adrenal gland is the endocrine organ most commonly involved at autopsy in patients who die of AIDS.[1,2] Adrenal dysfunction can increase morbidity and mortality among patients with HIV infection.[3] The clinical problem is to identify the patients with HIV disease who have impaired adrenocortical function and who either have or are at risk for adrenal insufficiency. Identification of adrenal insufficiency in the setting of HIV infection is imperative because treatment with corticosteroids can be life saving.[3,4] Signs and symptoms consistent with adrenal insufficiency in these patients include weight loss, anorexia, and nausea. Biochemical derangements include hyponatraemia and hyperkalaemia.

Adrenocortical insufficiency should be suspected in patients with otherwise unexplained symptoms and signs compatible with adrenal insufficiency, such as anorexia, nausea, weight loss, and fatigue, and especially in patients with more specific manifestations of adrenal insufficiency, such as postural hypotension, hyponatremia, or hyperkalemia.[3,4] ACTH studies in patients with HIV disease have detected sub-clinical alterations in cortisol levels to frank primary adrenal insufficiency especially in those without signs or symptoms suggestive of adrenal insufficiency. In these patients, baseline cortisol levels are typically normal or elevated.[8-13] Some patients (8% -14%) were found to have subnormal stimulation with ACTH in some studies.[5,6,8,9]

Adrenal insufficiency secondary to HIV infection has been well studied. Only a few cases of clinically significant primary adrenal insufficiency have been reported in persons with HIV infection, although a prevalence of 22% was reported by Piedrola G and his Co workers.[12] Freda and his colleagues found primary adrenal insufficiency in 6 hospitalized patients with AIDS.[13,14]

In the majority of HIV infected patients, weight loss, hyporexia, asthenia, nausea, vomiting, diarrhoea and dizziness, have been attributed to gastroenteritis and the effect of HIV infection and not due to adrenal insufficiency.[15] Reports of adrenal insufficiency secondary to HIV infection from Nigeria are scanty. Adadevoh BK reported that the plasma cortisol level in normal Nigerian adults was comparable to those obtained in European subjects.[16] Appropriate increase in serum cortisol to ACTH stimulation was also observed in normal Nigerian adults by the same author.[16] Adadevoh reported treating two cases of primary adrenal failure.[17]

It is in view of the non-consideration of adrenal insufficiency as a cause of the above symptoms, that it is important to detect the role of adrenal insufficiency in these categories of patients. This will further enhance the overall management of HIV infected patients. The objective of this study was to determine the prevalence of adrenocortical insufficiency in persons with HIV infection by determining the response to low-dose (1μg) ACTH stimulation.

METHODS
Consecutively presenting persons with HIV infection who met the inclusion criteria were selected. The estimated sample size of 42 was arrived at using the formula: $n = Z^2 \, pq/d^2$.[18] The estimated prevalence of HIV infection in Nigeria in 18-60 years age range is 5%[19] and the prevalence of adrenocortical insufficiency in persons with HIV infection was taken to be 50% since the prevalence is not known in Nigeria.[18]

Making allowance for dropouts, 50 HIV infected patients were recruited for the study groups and 100 patients were recruited for the control group to increase the power of the study.

Pregnant, diabetic and on individuals on medicines known to affect adrenocortical function were excluded from the study. Persons with HIV infection with concomitant pulmonary tuberculosis were also excluded from the study. Healthy HIV negative volunteers aged between 18 years and 60 years who consented to taking part in the study were recruited as control.

The study group and the controls were recruited from the Lagos University Teaching Hospital (LUTH). Informed consent was obtained from all subjects and the LUTH Ethics and Research Committee approved the study.

The study groups were divided into batches of 10 subjects each. A data collection sheet, filled by the investigator, was used to obtain information from the subjects and controls. Information obtained from each participant included the bio-data, presence of weakness, fatigue, cough, haemoptysis fever, weight loss, anorexia, nausea, vomiting, diarrhoea, a history of glucocorticoid and/or antiretroviral drug use.

The subjects arrived on the assigned day at the laboratory, 60 minutes before the ACTH testing, after an overnight fast of 8-10 hours. Physical examination including pulse rate and blood pressure in supine and erect position was performed. The anthropometric measurements (weight, height, waist circumference and hip circumference) were also taken. A 21-G canulla was inserted into a cubital vein and kept patent with heparinised saline. The subject then rested for 30 minutes after securing the venous assess before samples were collected.

Low dose short Synacthen® test was performed as follows. A baseline blood samples for cortisol, fasting plasma glucose (FPG), full blood count (FBC), ESR and electrolytes were collected immediately before administration of ACTH. ACTH testing was conducted between 08.00hour and 9.00hour. After the samples had been taken, the subject received an intravenous bolus injection of 1μg ACTH (Alliance Pharmaceuticals Ltd, Chippenham, Wiltshire SN15 2BB). After the bolus was administered, blood sample was drawn for cortisol level at 30 minutes. The samples were separated and transported on an ice slab to the laboratory where the plasma were stored at -20⁰C until assayed.

Data management
A normal basal cortisol, derived from 70 controls, was defined as a 0 minute cortisol level of ≥145.1nmol/L (mean - 3SD). A normal response to 1μg ACTH stimulation was defined as a 30-minute cortisol level of ≥380.2 nmol/L and an increment from basal to stimulated cortisol level of ≥158.5nmol/L. This was derived from 70 controls in which the minimum serum cortisol at 30-minute post ACTH stimulation was 380.2 nmol/L (mean-3SD) and minimum increment from basal to stimulated level was 158.5nmol/L (mean-3SD).
Using these values, adrenal insufficiency in this study was defined as 30-minute cortisol level <380.2nmol/L and increment from basal to stimulated cortisol level <158.5nmol/L.

Human Immunodeficiency Virus infection was diagnosed if screened positive by enzyme-linked immuno-sorbent assay (ELISA method) and confirmed by im-muno-electrotransference (Western blot).[20] Postural Hypotension was defined as difference between supine systolic blood pressure and erect systolic blood pressure >20mmHg.[21] A diagnosis of hypoglycaemia was made at plasma glucose level <2.3mmol/L.[22] A diagnosis of hyponatraemia was made at plasma sodium level <135mmol/L.[23] A diagnosis of hyperkalaemia was made at plasma potassium level >5.0mmol/L.[23]

A diagnosis of anaemia was made at haemoglobin level <12.0g/dL[24] Assay Serum cortisol levels were determined by an Enzyme Linked Immunosorbent Assay (ELISA) technique using the Diagnostic automation Inc. cortisol assay method. It is a competitive immuno-enzymatic colorimetric method for quantitative determination of cortisol concentration in serum. The respective intra-assay and inter-assay coefficient of variation of 4.5% and 3.1% for serum cortisol were within the acceptable range of variation.

Statistical Analysis
Calculations and analysis were done using the SPSS 15.0 software. Continuous variables were expressed as means ± standard deviation (SD). Student's t test was used for the comparison of means between two groups. Chi-square was used for comparison of proportions between two groups. The level of statistical significance was taken as p<0.05.

RESULTS
Demographic data
Forty-three persons with HIV completed the study while 7 persons with HIV infection declined stimulation with ACTH. There were 23 males and 20 females with mean ages of 41.5 ± 11.9 and 36.7 ± 11.2 years respectively (p=0.186). Of the one hundred healthy volunteers recruited as control, 70 persons (35 males and 35 females) completed the exercise.

Thirty persons declined further testing. There was no significant difference between male and females in the demographic and the anthropometric indices. However, the weight, BMI, waist circumference and haemoglobin were significantly lower in persons with HIV than in the control (Table 1).

The adrenal response to 1μg ACTH stimulation in persons with HIV infection and healthy subjects are is as shown in Table 2.

Table 1 Demographic data in subjects with HIV vs Healthy subjects

	HIV (n= 43)	Healthy Control (n= 70)
Age (years)	39.3±11.7	38.1±12.5
Weight (kg)	59.8±9.6*	65.9±11.1
BMI (kg/m^2)	21.7±3.5*	24.1±3.7
Waist Circumference (cm)	75.9±8.4*	81.4±9.0
Haemoglobin (g/dL)	10.8±2.1*	12.9±0.5
Supine systolic BP (mmHg)	116.7±09.4	119.4±12.9
Supine diastolic BP (mmHg)	73.9±06.9	76.2±10.4
Erect systolic BP (mmHg)	117.6±10.3	119.4±12.2
Erect diastolic BP (mmHg)	74.2±6.8	76.2±9.6

BMI; Body Mass Index. *p<0.05 (Control vs HIV)

The mean basal cortisol (0-minute) was 154.9±27.2 nmol/L while the 30-minute post ACTH test cortisol level was 354.8±19.9 nmol/L in HIV group. The basal cortisol level, 30-minute post ACTH tests cortisol level and increment was significantly lower in persons with HIV than healthy subjects.

Table 2 Comparison of Serum cortisol levels in response to ACTH test in healthy subjects and persons with HIV

Interval	Cortisol Level Mean ± SD nmol/L		p-value
	Healthy subjects	Persons with HIV	
0 minute	239.9 ± 31.6	154.9 ± 27.2	<0.001
30 minutes	870.9 ± 163.5	354.8 ± 19.9	<0.001
Increment	588.8 ± 143.4	100.0 ± 17.2	<0.001

Table 3 Frequency of Adrenocortical Insufficiency as seen in Persons with HIV Infection

Diagnostic Criteria (nmol/L) at 30 minutes post ACTH stimulation	Adrenocortical Response in Persons with HIV Infection (n=43)	
	Adrenocortical Insufficiency [n (%)]	Normal Response [n (%)]
Peak <380 + Incr. <158	15 (34.8)	28 (65.2)
<500	38 (83.4)	5 (11.6)

Using the cortisol values derived from healthy subjects, fifteen (34.8%) persons with HIV infection had adrenocortical insufficiency (Table 3). There was no significant difference between males and females.

The proportion of persons with HIV infection with normal and abnormal basal cortisol level is shown in Table 4. Comparison of a higher recommended cut-off value with that used in this Study is shown in Table 5.

Table 4 Proportions Of Persons With HIV Infection With Normal And Abnormal Basal Cortisol Level

Basal Cortisol level (nmol/L)	No of Persons with HIV [n (%)]
<145.1	2 (4.7)
≥145.1	41 (95.3)

Table 5 Comparison of a higher recommended cut-off value with that used in this study.

Gender	Adrenocortical response in persons with HIV Infection (n=43)	
	Adrenocortical Insufficiency [n (%)]	Normal Response [n (%)]
Male	8 (18.6)	15 (34.9)
Female	7 (16.2)	13 (30.3)
Both	15 (34.8)	28 (65.2)

Comparison of Biochemical parameters in persons with HIV and controls

The biochemical parameters in persons with HIV and healthy controls are compared in Table 6. The FPG was significantly lower in healthy subjects than in HIV group. The plasma sodium was significantly higher in the controls than in HIV group.

Table 6 Comparison of Plasma Biochemical Variables in persons with HIV and Healthy Subjects

Plasma Analyte	Mean ± SD		P-value
	HIV	Healthy subjects	
FPG(mmol/L)	5.2±0.9*	4.9±0.6	0.014
Na^+(mmol/L)	135.9±4.2*	138.1±1.5	0.026
K^+(mmol/L)	3.6±0.5	3.8±0.3	0.544
Urea(mmol/L)	3.7±2.8	3.2±0.5	0.199
Hb (g/dL)	10.8±2.1*	12.9±0.5	0.021

FPG, Fasting Plasma Glucose; Na^+, Sodium; K^+, Potassium; Hb, Haemoglobin.
*p<0.05 (Healthy subjects versus HIV).

Clinical Features of Adrenocortical Insufficiency in HIV Patients

The proportion of persons with HIV with clinical features of adrenocortical insufficiency is shown in Table 7. None of the patients with HIV had hyperpigmentation, postural hypotension or hypoglycaemia.

Table 7 Clinical Features of Adrenocortical Insufficiency in HIV Patients

Clinical Features	HIV [n (%)]
Weight loss	29 (67.4)
Weakness	10 (23.3)
Vomiting	1 (2.3)
Diarrhoea	3 (7.0)
Nausea	0 (0%)
Hyperpigmentation	0 (0%)
Postural hypotension	0 (0%)
Hypoglycaemia	0 (0%)

DISCUSSION

The adrenal glands play an important role in the body's ability to cope with stresses such as infections, hypotension and trauma including surgery.[25,26] The most common cause of primary adrenal insufficiency is tuberculous adrenalitis. Tuberculous adrenalitis is believed to be a major factor in the development of primary adrenal insufficiency in the developing world.[27] Another cause of primary adrenal insufficiency is the HIV infection, in which the adrenal gland may be destroyed either by the virus itself or a variety of opportunistic infectious agents in up to 5% of patients in the late stages of the HIV infection.[3,28] The adrenal cortex is a common site of pathological involvement in patients infected with the human immunodeficiency virus (HIV) and the acquired immune deficiency syndrome (AIDS) that results from it.[29] Detection of impaired adrenocortical function is of potential relevance as cortisol deficiency could account for unexpected deaths seen occasionally in patients with HIV.[30]

Our study demonstrated that the mean basal cortisol value (154.9nmol/L) and 30-minute cortisol value (354.8nmol/L) in persons with HIV was significantly lower than mean basal and 30 minute cortisol levels in the healthy controls. The mean incremental rise (100nmol/L) was also significantly lower in persons with HIV. Using the diagnostic criteria for adrenocortical insufficiency in this study, (peak cortisol <380nmol/L and increment <158.5nmol/L), we found that 15 (34.8%) persons out of 43 with HIV had adrenal insufficiency (Table 3).

However, using serum cortisol level of 500nmol/L suggested as the cut-off point for 30 minute cortisol response [31], 38 persons (88.4%) out of 43 persons with HIV had subnormal response to ACTH test in this study (Table 4). This might be an over estimation of the prevalence of adrenocortical failure in persons with HIV infection in the study, hence the use of a stringent diagnostic criteria in this study. Some studies have reported normal or elevated basal cortisol levels in persons with HIV infection.[6,8,9]

In this study, most of the persons with HIV infection had normal basal cortisol (Table 4). This is similar to the finding of Raffi et al[9]. The adrenocortical response to ACTH stimulation was abnormal in 34.8% of persons with HIV in this study. This is similar to the findings in previous studies.[6,8,9,11] Gonzalez et al found adrenal insufficiency in 21% of cases with HIV.[32] This, however, is slightly lower than the value in this study. The cut-off point for adrenocortical insufficiency was set at peak cortisol level <607nmol/L in the study by Gonzalez et al. The lower percentage of persons with HIV with adrenocortical insufficiency in this study cannot be easily explained. However a possible explanation lies in the fact that they used 10 microgram of ACTH in their study. One microgram ACTH used in this study had been shown to have better sensitivity.[33,34]

The abnormal response may be attributable to minimal degree of adrenal damage recognised in cases from autopsy studies.[1,35] The amount of adrenal gland tissue remaining functional, however, is apparently enough to provide a satisfactory glucocorticoid production in the basal state. In times of stress, adrenocortical response may not be adequate. None of the patients with HIV had hyper pigmentation. This was similar to the finding in a previous study.[36] There was also no postural drop in blood pressure measurement in those with impaired adrenal response to ACTH test. Postural hypotension is a sign of volume depletion. This is similar to the finding by Kaplan et al [36]. The mean systolic or diastolic blood pressure was comparable in the HIV and the healthy control groups and also in those with impaired serum cortisol response. This is in keeping with studies done in Kenya and South Africa.[27,36,37]

The mean fasting plasma glucose is significantly different in persons with HIV and healthy control. However it is within the acceptable normal range. Those with impaired cortisol response had no record of hypoglycaemia. This is similar to the findings in previous studies.[27,36,37] Each of these studies found glucose concentration to be similar in those with an impaired cortisol response and those with normal response. The normoglycaemia seen in them is explained by their normal basal cortisol level.[1,35]

Of the 12 persons with HIV who had hyponatraemia, only 2 had impaired 30-minute cortisol response to ACTH. Hyponatraemia is usually due to syndrome of inappropriate antidiuretic hormone secretion rather than adrenal insufficiency.[13,38] None of the person with HIV infection had the classical hyperkalaemia seen in adrenal insufficiency. This is similar to findings in other studies in other parts of Africa.[36,39] The mineralocorticoids are under the control of the renin angiotensin system. This system is probably intact in people with HIV infection. Furthermore, the functional gland remaining is adequate for mineralocorticoid production.[1,35] Clinically evident adrenocortical insufficiency is uncommon in persons with HIV.

Most persons with HIV infection in this study had normal basal cortisol level, had no classical features of adrenocortical insufficiency. It was however apparent from the dynamic stimulation of their adrenal gland with ACTH that the adrenal response was impaired. Adrenocortical dysfunction is a potentially life-threatening condition that may affect about 34.8% of persons with HIV. This diagnosis should be considered whenever any of these groups of people presents with unexplained symptoms and signs compatible with adrenal insufficiency, such as anorexia, nausea, weight loss, and fatigue, and especially in patients with more specific manifestations of adrenal insufficiency such as postural hypotension, hyponatremia, or hyperkalemia on the background of stressful conditions such as trauma and infection.

CONCLUSION

Basal serum cortisol levels are within the normal range in many persons with HIV infections even in the presence of reduced adrenocortical function. Adrenocortical insufficiency, mostly at the subclinical level, is common in persons with HIV infection, occurring in about 34.8% of patients.

We therefore recommend that basal cortisol levels should not be used to elicit adrenocortical insufficiency; rather stimulation tests should be used to exclude or confirm suspected adrenocortical insufficiency in persons with HIV. We also recommend that corticosteroids should not be routinely used in persons with HIV. However, corticosteroid use may be indicated when these persons with biochemical evidence of subclinical adrenocortical insufficiency are exposed to stressful conditions like surgery, infections or trauma.

Limitations

A potential limitation to the study is the use of lower cut-off value for the diagnosis of adrenocortical insufficiency. This could have underestimated the proportion of persons with HIV infection with adrenocortical insufficiency. Dilution error could be a potential source of error. It would have been ideal if there is commercially prepared 1ug ACTH.

REFERENCES

1. Bricaire F, Marche C, Zoubi D, Regnier B, Saimot AG. Adrenocortical lesions and AIDS.*Lancet.*1988; 1:881.

2.   Welch K, Finkbeiner W, Alpers CE, et al. Autop-sy findings in the acquired immune deficiency syndrome. *JAMA* 1984; 252:1152–9.

3.   Eledrisi MS, Verghese AC. Adrenal Insufficiency in HIV Infection: A Review and Recommenda-tions *Am J Med Sci* 2001; 321(2):137–144.

4.   Lo J, Grinspoon SK. Adrenal function in HIV infection. *Current Opinion in Endocrinology, Di-abetes & Obesity* 2010, 17:205–209

5.   Dobs AS, Dempsey MA, Ladenson PW, Polk BF: Endocrine disorders in men infected with human immunodeficiency virus. *Am J Med* 1988; 84:611-616.

6.   Membrano L, Irony I, Dere W, Klein R, Biglieri EG, Cobb E: Adrenocortical function in acquired immunodeficiency syndrome. *J Clin Endocrinol Metab* 1987; 65:482-487.

7.   Verges B, Chavanet P, Desgres J, Vaillant G, Waldner A, Brun JM et al: Adrenal function in HIV infected patients. *Acta Endocrinol (Copenh)* 1989; 121:633-637.

8.   Villette JM, Bourin P, Doinel C, Mansour I, Fiet J, Boudou P et al: Circadian variations in plasma levels of hypophyseal, adrenocortical and testicu-lar hormones in men infected with human immu-nodeficiency virus. *J Clin Endocrinol Metab* 1990; 70:572-577.

9.   Raffi F, Brisseau JM, Planchon B, Rémi JP, Bar-rier JH, Grolleau JY: Endocrine function in 98 HIV-infected patients: A prospective study. *AIDS* 1991; 5:729-733.

10.   Christeff N, Gharakhanian S, Thobie N, Rozen-baum W, Nunez EA: Evidence for changes in ad-renal and testicular steroids during HIV infection. *J Acquir Immune Defic Syndr* 1992; 5:841-846.

11.   Peter SA, Bruschetta H, Vergara R: Glucocorti-coid reserve in patients with acquired immunode-ficiency syndrome. *Horm Res* 1995; 44:85-88.

12.   Piédrola G, Casado JL, López E, Moreno A, Pe-rez-Elías MJ, Garcia-Robles R: Clinical features of adrenal insufficiency in patients with acquired immunodeficiency syndrome. *Clin Endocrinol* 1996; 45:97-101.

13.   Freda PU, Wardlaw SL, Brudney K, Goland RS. Primary adrenal insufficiency in patients with the Acquired immunodeficiency sydrome: a report of five cases. *J Clin Endocrinol Metab*. 1994; 79:1540-1545.

14.   Freda PU, Papadopoulos AD, Wardlaw SL, Go-land RS: Spectrum of adrenal dysfunction in pa-tients with acquired immunodeficiency syndrome: Evaluation of adrenal and pituitary reserve with ACTH and corticotropin-releasing hormone test-ing. *Trends Endocrinol Metab* 1997; 8:173-180.

15.   Watson JP, Lewis RA. Schmidt's syndrome asso-ciated with sarcoidosis. *Postgrad Med J* 1996; 72:435-**436**

16.   Adadevoh BK. Adrenocortical function in Nigeri-ans. *The W Afr Med J* 1970; 19:25-26.

17.   Adadevoh BK. Endocrine patterns in the African. *Trop Geogr.Med* 1970; 22:125-141.

18.   Araoye MO. Research Methodology with statis-tics for health and social sciences. Ilorin: Nathadex, 2003; 90-95.UNAIDS, Epidemiologi-cal fact sheet Nigeria, update 2004.

19.   CDC. Revision of the CDC surveillance case def-inition for acquired immunodeficiency syndrome. MMWR 1987; 36:1-15S.

20.   International Society of Hypertension Guidelines for the Management of Hypertension. WHO 1999.

21.   Cryer PE: Glucose Homeostasis and Hypogly-caemia. In Larson PR, Kroneberg HM, Melmed S. Polonsky KS [eds]. Williams textbook of Endo-crinology. Philadelphia. Saunders. 2003: 1585-1599.

22.   Mayne PD: Sodium and Potassium Metabolism. Clinical Chemistry in Diagnosis and treatment. United Kingdom. ELBS. 1994:26-78.

23.   World Health Organization. Worldwide preva-lence of anaemia 1993–2005. Geneva, World Health Organization, 2008

24.   Miller WL, Chrousos GP. The adrenal cortex. In: Felig P, Frohman LA eds: *Endocrinology and Metabolism.* 4th edn USA: McGraw-Hill.2001:387-524.

25.   Oelkers W: Adrenal Insufficiency. *N. Engl. J Med* 1996; 335:1206-1212.

26.   Soule S. Addison's disease in Africa: A teaching hospital Experience. *Clin Endocrinol* 1999; 50:115-120.

27.   Oelkers W, Diederich S,Bahr V. Recent advances in diagnosis and therapy of addison's disease. In: Bhatt HR, James VHT, Besser GM eds. Advances in Thomas Addison's disease. Vol 1.London: Thomas Addison's society, 1994; 69-80.

28.   Aron DC: Endocrine complications of the ac-quired immunodeficiencv svndrome. *Arch Intern Med*. 1989; 149:330-333.

29.   Baker RW, Zumla A, Rook GAW. Tuberculosis, steroid metabolism and immunity. *Q J Med* 1996; 89: 387-394.

30.   Stewart PM: The adrenal cortex. In Larson PR, Kroneberg HM, Melmed S. Polonsky KS [eds]. *William textbook of Endocrinology. Philadelphia. Saunders.* 2003: 491-585.

31.   Gonzalez JG, Garza-Hernandez NE, Garza-Moran RA, Rivera-Morales IM, Montes-Villarreal J, Valenzuela-Rendon J et al: Prevalence of abnor-mal adrenocortical function in human immunode-

ficiency virus infection by low-dose cosyntropin test. *International Journal of STD & AIDS* 2001; 12:804-810.

32. Daidoh H, Morita H, Mune T, Murayama M, Hanafusa J, Ni H, Shibata H & Yasuda K . Responses of plasma adrenocortical steroids to low dose ACTH in normal subjects. *Clinical Endocrinology* 1995; 43: 311–315.

33. Dickstein G, Spigel D, Arad E, Shechner C. One µg is the lowest ACTH dose to cause maximal cortisol response, unrelated to weight. There is no diurnal variation of cortisol response to submaximal ACTH stimulation. *Eur J Endocrinol* 1997; 137:172-175.

34. Glasgow BJ, Steinsapir KD, Anders K, Layfield LJ: Adrenal pathology in the acquired immune deficiency syndrome. *Am J Clin Pathol* 1985; 84:594-597.

35. Kaplan FJL, Levitt NS, Soule SG: Primary hypoadrenalism assessed by the 1µg ACTH test in hospitalized patients with active pulmonary tuberculosis. *Q J Med* 2000; 93: 603-609.

36. Hawken MP, Ojoo JCO, Morris JS, Kariuki EW, Githui WA, Juma ES: No increased prevalence of adrenocortical insufficiency in human immunode-

ficiency virus-associated tuberculosis. *Tubercle and Lung Disease* 1996; 77: 444—448.

37. Cusano AJ, Thies HL, Siegel FP, Dreisbach AW, Maesaka JK: Hyponatraemia in patients with acquired immune deficiency syndrome. *J Acquir Immune Defic Syndr* 1990; 3: 949-953.

38. Post FA, Soule SG, Willcox PA, Levitt NS: The spectrum of endocrine dysfunction in active pulmonary tuberculosis. *Clin Endocrinol 1994; 40: 367-371.*Ellis H. Intestinal obstruction. New York; Appleton- Century and Crofts, 1982: 175

39. Grimes CE, Law RSL, Borgstein ES, Mkandawire NC, Lavy CBD. Systematic review of met and unmet needs of surgical disease in rural sub-Saharan Africa. *World J Surg* 2012; 36: 8-23.

40. Beard JH, Oresanya LB, Ohene-Yeboah M, Dicker RA, Harris HW. Characterizing the global burden of surgical disease: A method to estimate inguinal hernia epidemiology in Ghana *World J Surg* 2013;37(3): pg 498-503

41. Abantanga FA, Hesse AJ, Sory E, Osen H, Choo S, et al. Policies for improving access to and quality of essential basic surgical care at district hospitals in Ghana. *Post Grad Med J Ghana* 2012; 1(1): 3-8 ✪