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Serum Sex Hormonal Levels In Malaria Infected Symptomatic HIV (Stage 11) Male Subjects On Antiretroviral Therapy (Art) In Nnewi, South Eastern, Nigeria

Prof. Ahaneku J.E
Department of Chemical Pathology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State,Nigeria.

Dr. Onyenekwe C.C
Department of Chemical Pathology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State,Nigeria.

Prof. Meludu S.C
Department of Human Biochemistry, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State,Nigeria.

Mrs Ezeugwunne I.P.
Department of Human Biochemistry, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, AnambraState, Nigeria.

Dr. Ifeanyichukwu M
Department of Immunology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State,Nigeria.

Mr Nnadozie O.J
Department of Chemical Pathology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria

Abstract:

The present study was designed to determine the impact of HIV and malaria co-infection on the serum hormonal levels of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone, Estrogen, Progesterone, Prolactin and Cortisol symptomatic HIV seropositive male participants on ART. A total of 275 adult participants aged between 18 and 60 years were randomly recruited for this study. The participants were enlisted based on World Health Organisation (WHO) criteria. 139 symptomatic HIV infected participants on ART of which 70 of them had malaria co-infection and 136 HIV seronegative control participants of which 68 of them had malaria co-infection. Blood samples were collected from the participants for the determination of HIV status by Immunoassay. Thick and thin films prepared from the blood samples were examined and malaria parasites density was counted according to the World Health Organisation (WHO) recommendation. Also, ELISA method was used to assay for serum sex hormones. The result showed that the serum LH, FSH, Progesterone and Estrogen levels were significantly higher in malaria infected and malaria uninfected symptomatic HIV participants on ART (in each case) compared with malaria infected and malaria uninfected HIV seronegative control participants ($p<0.05$). The mean Testosterone and Prolactin levels also showed significantly lower values in malaria infected and malaria uninfected symptomatic HIV participants on ART compared with malaria infected and malaria uninfected HIV seronegative control participants ($p<0.05$). The mean Cortisol levels were similar amongst the groups studied ($p>o.05$, in each case). Similarly, the malaria parasite density (MPD) was significantly higher in malaria infected compared with malaria uninfected symptomatic HIV participants on ART participants ($p<0.05$). The implication of this finding is that HIV and malaria co-infection has an effect on some of these sex hormones on participants on ART.

Keyword*: HIV, malaria, antiretroviral therapy and sex Hormones.*

Introduction

Malaria and HIV-1 and 2 are the most common infections in the sub-Sahara Africa. Researchers have observed co-morbidity in both pathogens in Africa [1,2,3]. The existence of HIV and malaria co-infection has been reported in South Eastern Nigeria [4]. Onyenekwe *et al* [4] in their work observed almost a triple-fold prevalence of *Plasmodium falciparum* in symptomatic HIV participants that were infected with the malaria disease. Nevertheless, HIV disease is diminished with effective use of antiretroviral therapy [5,6]

The endocrine reproductive system produces reproductive hormones such as FSH, LH, Testosterone, Estrogen, Progesterone, Prolactin and Cortisol into the general circulation [7,8,9]. A number of endocrine abnormalities have been developed in patients with HIV infection [10]. Hypogonadism has been found to be on increase in HIV dIsease [11]. The most common endocrine abnormality was found to be low serum Testosterone level in symptomatic HIV infection [11]. Also, most HIV infected person has been reported to have elevated serum Cortisol [121]., elevated serum LH [13] and elevated serum FSH [13,14]. Males have been reported to be more susceptible to malarial infection than female [15]. This increased susceptibility to malarial infection was linked with circulating steroid hormones [16,17,18]. Testosterone level has been found to have a positive correlation with malaria parasitemia in adult male subjects, in Honduras [19,20]. These workers also reported lower Testosterone levels and significantly higher Cortisol levels in males infected with *Plasmodium vivax* parasitemia than in healthy individuals. Hence, the reason for this study is to evaluate the effect of HIV and / or malaria on the sex hormones in symptomatic HIV (stage 11) infected participants on ART in South Eastern Nigeria since this subject of study has not been done in South Eastern Nigeria.

Materials And Methods

Subjects

A total of 275 adult male participants aged between 18 and 60 (42 ±13) years were randomly recruited at both Antiretroviral Therapy (ART) Clinic and Voluntary Counseling and Testing (VCT) Centre in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria. The participants were classified based on WHO criteria for HIV staging. 139 symptomatic HIV (stage 11) infected participants on ART (Lamivudine, Stavudine and Nevirapine) of which 70 of them had malaria co-infection and 136 HIV seronegative control participants of which 68 of them had malaria co-

infection. 5 ml of blood samples were collected from each of the participant and dispensed into EDTA tube for HIV screening by Immunoprecipitation and Immunochromatographic method. The malaria parasite density count was examined microscopically by thick and thin film method. The serum from the remaining blood placed into a plain tube was used for the estimation of FSH, LH, Testosterone, Estrogen, Progesterone, Prolactin and Cortisol levels by ELISA method. The serum samples were stored at -20°C until analysed. Informed consent was obtained from those who participated in the study. The Nnamdi Azikiwe University Teaching Hospital Board of Ethical Committee approved the study design.

Methods

Detection of Antibodies to HIV-1 and HIV-2 in Human plasma

Two different methods were used, namely, Abbott determine ™ HIV -1 and HIV-2 kit, which is an in-vitro visually read immunoassay (Abbott Japan Co.Ltd.Tokyo, Japan) and HIV-1 and 2 STAT-PAK Assay kit, which is an Immunochromatographic test for the quantitative detection of antibodies to HIV-1 and HIV-2 in Human plasma (CHEMBIO Diagnostic system, Inc, New York, USA). For the Abbott determine ™ HIV -1 and HIV-2 kit, the procedure described by the manufacturer was used for the analysis. Briefly, 50 µl of participant serum samples separated from the corresponding whole blood samples in EDTA were applied to the appropriately labeled sample pad. After 15 minutes but not more than 60 minutes of the sample application, the result was read. This method has inherent quality control that validates the results. For the Immunochromatographic method for HIV -1 and HIV-2, the procedure described by the manufacturer was used for the analysis. In brief, 5 ml of participant's plasma was dispensed into the sample well in the appropriately labeled sample pad. Three drops of the buffer supplied by the manufacturer was added into the appropriately labeled sample pad. The results of the test were read at 10 minutes after the addition of the running buffer. This method has inherent quality control and validates the results.

Thick and thin film as will be described by WHO [21]

Thick and thin films will be prepared for each participant's blood sample. The thin films were fixed with methanol and both thick and thin films were stained with Giemsa (1 in10 dilution) for 10 minutes, after which they were examined microscopically with oil immersion (x 100) objective. The malaria parasite counting was done using the thick

blood films while the thin blood films were used for species identification. Malaria parasites were counted according to the method of World Health Organisation [21]. 200 leukocytes were counted and if 10 or more parasites were identified, then the number of parasites per 200 leukocytes was recorded; but if after counting 200 leukocytes and 9 or less parasites identified then, 500 leukocytes was recorded. In each case the parasite count in relation to the leukocyte count was converted to parasite per microlitre of blood using this mathematical formula:

$$\text{Malaria parasite density } /\mu l = \frac{\text{number of parasites x } 8000}{\text{Number of leukocytes}}$$

Where 8000, is the average number of leukocytes per microlitre of blood, which is taken as the standard (WHO, 1995).

CD4$^+$ T CELL Count by Flow Cytometry

200 ml EDTA whole blood was collected into PARTEC test tubes (Rohren tube). Then 20 µl of CD4$^+$ T antibody was added into the tube. The contents were mixed and incubated in the dark for15 minutes at room temperature. 800 ml of CD4 buffer was gently added into the mixture and mixed gently. Then the Partec tube was plugged on the Cyflow counter and the CD4$^+$ T cells were displayed as peaks and interpreted as figures.

Estimation of LH by Randox Laboratories Limited, U.K.

Serum LH was estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK); 50 µl of serum sample was added to appropriately labeled microtitre wells and 100 µl Enzyme conjugated detection antibody was also added to the wells. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated at room temperature for 45 minutes. The wells were washed 3 times with deionized water to remove unbound antibodies. 100µl TMB reagent was added and well incubated in the dark for 20 minutes for the colour to develop. 100µl of HCL was added to the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density values and concentrations

of series of FSH standards (0, 5, 15, 50, 100 and 200 miu/ml) provided by the manufacturer of the kit.

Estimation of FSH by Randox Laboratories Limited, U.K.

Serum FSH was estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK); 50 µl of serum sample was added to appropriately labeled microtitre wells and 100 µl Enzyme conjugated detection antibody was also added to the wells. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated at room temperature for 45 minutes. The wells were washed 3 times with deionized water to removed unbound antibodies. 100µl tetramethylbenzidine (TMB) reagent was added and well incubated at room temperature for 20 minutes for the colour to develop. 100µl of hydrochloric acid (HCL) was added to the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density (OD) values and concentrations of series of FSH standards (0, 5, 15, 50, 100 and 200 miu/ml) provided by the manufacturer of the kit.

Estimation of Progesterone by Randox Laboratories Limited, U.K.

Serum Progesterone was estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK); 25 µl of serum sample was added to appropriately labeled microtitre wells and 100 µl Progesterone Enzyme horseradish peroxidase conjugate reagent was added to the wells. Also, 50µl of rabbit anti-Progesterone reagent was added in each well. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated at room temperature for 90 minutes. The wells were washed 3 times with deionized water to removed unbound antibodies. 100µl TMB reagent was added and well incubated at room temperature for 20 minutes for the colour to develop. 100µl of HCL was added to the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density values and

concentrations of series of FSH standards (0, 0.5, 3, 10, 25 and 50 ng/ml) provided by the manufacturer of the kit.

Estimation of Testosterone by Randox Laboratories Limited, U.K.

Serum Testosterone was estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK); 10 µl of serum sample was added to appropriately labeled microtitre wells and 100 µl Enzyme conjugated detection antibody was also added to the wells. Also, 5µl of rabbit anti-Testosterone reagent was added in each well. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated at 37°C for 90 minutes. The wells were washed 3 times with deionized water to removed unbound antibodies. 100µl TMB reagent was added and well incubated at room temperature for 20 minutes for the colour to develop. 100µl of HCL was added to the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density values and concentrations of series of FSH standards (0, 0.1, 0. 5, 2, 6 and 18 ng/ml) provided by the manufacturer of the kit.

Estimation of Prolactin by Randox Laboratories Limited, U.K.

Serum Prolactin was estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK); 50 µl of serum sample was added to appropriately labeled microtitre wells and 100 µl Enzyme conjugate reagent was also added to the wells. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated at room temperature for 45 minutes. The wells were washed 3 times with deionized water to removed unbound antibodies. 100µl TMB reagent was added and well incubated at room temperature for 20 minutes for the colour to develop. 100µl of HCL was added to the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density values and concentrations of series of FSH standards (0, 5, 15, 50, 100 and 200 ng/ml) provided by the manufacturer of the kit.

Estimation of Estradiol by Randox Laboratories Limited, U.K.

Serum Estradiol was estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK); 25 µl of serum sample was added to appropriately labeled microtitre wells and 100 µl Enzyme conjugated detection antibody was also added to the wells. Also, 5µl of rabbit anti- Estradiol reagent was added to each well. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated at 37°C for 90 minutes. The wells were washed 3 times with deionized water to removed unbound antibodies. 100µl TMB reagent was added and well incubated at room temperature for 20 minutes for the colour to develop. 100µl of HCL was added to the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density values and concentrations of series of FSH standards (0, 10, 30, 100 and 1000 pg/ml) provided by the manufacturer of the kit.

Estimation of Cortisol by Randox Laboratories Limited, U.K.

Serum Cortisol was estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK); 20 µl of serum sample was added to appropriately labeled microtitre wells and 200 µl Enzyme conjugate reagent was also added to the wells. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated at room temperature for 60 minutes. The wells were washed 3 times with deionized water to removed unbound antibodies. 100µl TMB reagent was added and well incubated at room temperature for 20 minutes for the colour to develop. 100µl of HCL was added to the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density values and concentrations of series of FSH standards (0, 20, 50, 100, 200, 400 and 800 ng/ml) provided by the manufacturer of the kit.

Statistical analysis

The result of the analysis was statistically analysed. Students't-test and one way analysis of variance (ANOVA) were used to compare means. The analysis was performed with the use of Statistical *Package for Social Sciences* (SPSS) statistical software package, version 13.0. $P < 0.05$ was considered statistically significant.

Result:

Variables	MPD/µl	LH (miu/ml)	FSH (miu/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)	Prolactin (ng/ml)	Estrogen (pg/ml)	Cortisol (ng/ml)
Symptomatic HIV stage 11 participants on ART with malaria infection (n=70)	708.50± 337.82	14.70± 6.71	16.02± 7.39	1.44± 1.14	2.71± 2.24	6.13± 3.07	51.30± 25.38	139.98± 63.45
Symptomatic HIV stage 11 participants on ART without malaria infection (n=68)		12.02± 7.45	14.38± 7.96	2.42± 1.73	2.58± 1.58	7.15± 5.33	58.99± 45.58	116.04± 74.45
HIV seronegative participants with malaria infection (n=69)	538.97± 351.15	9.33 ± 6.06	8.54± 5.25	1.14± 0.93	3.37± 2.24	8.49± 3.25	51.10± 37.68	147.70± 63.57
HIV seronegative participants without malaria infection (n=68)		9.56± 7.16	11.53± 7.58	1.09± 1.19	4.80± 1.75	9.39± 3.34	31.14± 18.20	130.02± 68.22
F (p) value	7.45 <0.05	9.27 <0.05	14.65 <0.05	16.12 <0.05	25.73 <0.05	9.58 <0.05	13.50 <0.05	2.89 >0.05
tIp[a]	----	0.128	0.592	0.001	0.999	0.512	0.612	0.241
tIp[b]	----	0.000	0.000	0.324	0.000	0.000	0.001	0.977
tIp[c]	----	0.000	0.003	0.292	0.000	0.000	0.000	0.938
tIp[d]	0.007	0.000	0.000	0.000	0.000	0295	0.001	0.041
tIp[e]	----	0.000	0.003	0.000	0.000	0.020	0.000	0.640
tIp[f]	----	0.970	0.043	0.242	0.985	0.378	0.003	0.375

Table 1: Mean (±SD) serum sex hormonal levels in malaria infected and malaria uninfected male symptomatic HIV (stage 11) participants on ART and in malaria infected and malaria uninfected HIV seronegative participants

Key

F (p) value = Symptomatic HIV stage (11) on ART with and without malaria infection and HIV seronegative with and without malaria infection all compared (using ANOVA).

tIp^a = Symptomatic HIV stage 11 on ART with and without malaria infection compared (using student's t-test).

tIp^b = Symptomatic HIV stage 11 on ART with malaria infection compared with HIV seronegative with malaria infection (using student's t-test).

tIp^c = Symptomatic HIV stage 11 on ART with malaria infection compared with HIV seronegative without malaria infection (using student's t-test).

tIp^d = Symptomatic HIV stage 11 on ART without malaria infection compared with HIV seronegative with malaria infection (using student's t-test).

tIp^e = Symptomatic HIV stage 11 on ART without malaria infection compared with HIV seronegative without malaria infection (using student's t-test).

tIp^f = HIV seronegative with malaria infection compared with HIV seronegative without malaria infection (using student's t-test).

The mean (±SD) LH (miu/ml), FSH (miu/ml), Progesterone (ng/ml) and Estrogen (pg/ml) serum concentrations were significantly higher in both symptomatic HIV stage 11 on ART with and without malaria infection compared with the values in HIV seronegative with and without malaria infection ($p<0.05$). Similarly, the serum Testosterone (ng/ml) and Prolactin (ng/ml) concentrations were significantly high in HIV seronegative with and without malaria infection compared with the values in symptomatic HIV stage 11 on ART with and without malaria infection ($p<0.05$). However, mean (±SD) Cortisol (ng/ml) serum concentration compared among the four showed no significant difference ($p>0.05$). See table 1.

Within group comparison showed that mean (±SD) serum concentration of LH, 14.70 (±6.71), FSH, 16.02 (±7.39) and Estrogen, 51.30 (±25.38) were significantly higher in the symptomatic HIV stage 11 on ART with malaria infection compared with the value LH, 9.33 (±6.06), FSH, 8.54(±5.25) and Estrogen, 51.10 (±37.68) in HIV seronegative with malaria infection ($p<0.05$). The mean (±SD) serum Testosterone, 3.37(±2.24) and Prolactin, 8.49 (±3.25) were significantly high in HIV seronegative with malaria infection compared with the values Testosterone, 2.71 (±2.24) and Prolactin, 6.13 (±3.07) symptomatic HIV stage (11) on ART with malaria ($p<0.05$, in each case).

However, mean (±SD) serum Progesterone compared between the symptomatic HIV stage 11 on ART with malaria and HIV seronegative with malaria infection showed similar values.

The mean (±SD) serum Progesterone, 2.42 (±1.73) in symptomatic HIV stage (11) on ART without malaria infection was significantly higher compared with mean (±SD) of Progesterone, 1.44 (±1.44) in symptomatic HIV stage (11) on ART with malaria ($p<0.05$). However, the mean (±SD) serum LH, FSH, Testosterone, Prolactin and Estrogen compared in each case between symptomatic HIV stage 11 on ART without malaria infection and the symptomatic HIV stage (11) on ART with malaria infection showed similar value ($p>0.05$, in each case).

The mean (±SD) serum concentration of LH, 14.70 (±6.71), FSH, 16.02 (±7.39) and Estrogen, 51.30 (±25.38) in symptomatic HIV stage 11 on ART with malaria infection was significantly higher than the mean LH, 9.56 (±7.16), FSH, 11.53 (±7.58) and Estrogen, 31.14 (±18.20) in HIV seronegative participants with malaria infection ($p<0.05$). Also, the mean (±SD) Testosterone, 4.80 (±1.75) and Prolactin, 9.39 (±3.34) in HIV stage 11 on ART without malaria infection showed higher significant difference compared with the values in Testosterone, 2.71 (±2.24) and Prolactin, 6.13 (±3.07) in the symptomatic HIV stage 11 on ART with malaria infection ($p>0.05$, in each case). However, the mean (±SD) serum concentration of Progesterone compared between the symptomatic HIV stage 11 on ART with malaria infection and HIV seronegative without malaria infection showed no significant difference ($p>0.05$).

The mean (±SD) serum concentration of LH, 12.02 (±7.45), FSH, 14.38 (±7.96), Progesterone, 2.42 (±1.73) and Estrogen, 58.99 (±45.58) showed significantly levels compared with mean values of LH, 9.33 (±6.06), FSH, 8.54 (±5.25), Progesterone, 1.14 (±0.93) and Estrogen, 51.10 (±37.68) in HIV seronegative participants with malaria infection ($p<0.05$, in each case).

Also, the mean (±SD) serum concentration of Testosterone, 3.37 (±2.24) in HIV seronegative participants with malaria infection was significantly higher compared with the value 2.58 (±1.58) in symptomatic HIV stage 11 on ART without malaria infection ($p<0.05$). However, the mean (±SD) serum concentration of Prolactin in symptomatic HIV stage 11 on ART without malaria infection compared with HIV seronegative participants with malaria infection showed similar mean values ($p>0.05$).

The mean (±SD) LH, 12.02 (±7.45), FSH, 14.38 (±7.96), Progesterone, 2.42 (±1.73) and Estrogen, 58.99 (±45.58) concentration in symptomatic HIV stage 11 on ART

without malaria infection were significantly higher compared with mean values of LH, 9.56 (±7.16), FSH, 11.53 (±7.58), Progesterone, 1.19 (±1.09) and Estrogen, 31.14 (±18.20) in the HIV seronegative participants without malaria infection ($p<0.05$, in each case). Also, the table showed that mean (±SD) Testosterone, 2.58 (±1.58) and Prolactin, 7.15 (±5.33) serum concentrations in symptomatic HIV stage 11 on ART without malaria infection showed lower levels compared with the mean values Testosterone, 4.80 (±1.75) and Prolactin, 9.39 (±3.34) in HIV seronegative participants without malaria infection ($p<0.05$, in each case).

The mean (±SD) FSH, 8.54 (±5.25) concentration in HIV seronegative participants with malaria infection is significantly lower compared with the FSH, 11.53 (±7.58) in HIV seronegative participants without malaria infection ($p<0.05$).

The mean (±SD) Estrogen 51.10 (±37.68) concentrations in HIV seronegative participants with malaria infection showed higher levels compared with the mean Estrogen31.14 (±18.20) in HIV seronegative participants without malaria infection ($p<0.05$). However, mean LH, Progesterone, Testosterone and Prolactin compared between HIV seronegative participants with and without malaria infection showed similar statistical values ($p>0.05$, in each case).

Discussion

The present study revealed a significantly increased value in the malaria parasite density (MPD) in malaria infected HIV stage 11 participants on ART. The increased MPD observed on these participants, might be as a result of the effect of malaria on the already compromised state of the host system. *Plasmodium falciparum* have been shown to stimulate HIV replication through the production of cytokines by activated lymphocytes [22] and also, HIV related immunosuppression has been suggested to increase the rate of malaria infection and these facts might be the cause of the increase in both HIV viral load and malaria parasite density.

In this study, there was no significant different observed in serum LH level between malaria infected and malaria uninfected HIV stage 11 participants on ART. But a higher LH value was observed in participants with malaria infection. This confirms the fact that HIV infection increases the rate of malaria infection in the Immunocompromised participants (Laufer *et al,* 2006). Similarly, there was no significant different observed in serum FSH level between malaria infected and malaria uninfected HIV (stage 11 participants on ART. Also, a higher FSH value was observed on participants with

malaria infection. This confirms the fact that HIV infection increases the rate of malaria infection in the Immunocompromised participants [23]. The Progesterone value was significantly higher in malaria uninfected HIV stage 11 participants on ART than in malaria infected HIV stage 11 participants on ART. This implies that the antiretroviral therapy reduces the susceptibility of the host system to be prone to malaria parasite infection.

In this study, serum low Testosterone value was significantly reduced in malaria infected and malaria uninfected HIV stage 11 participants on ART. This may be due to the effect of HIV on the gonads [11]. Also, The Testosterone value was higher in malaria infected HIV stage 11 participants on ART. This implies that HIV infection increases the rate of malaria infection in the Immunocompromised participants [23].

In this study, serum low Prolactin value was observed in malaria infected HIV stage 11 participants on ART than on participants without malaria infection. This implies that ART has a stabilizing effect on the endocrine glands and immune recovery of HIV stage 11 participants on ART hence the reduction in the rate of malaria infection in the Immunocompromised HIV participants.

The study further revealed no malaria effect on the release of serum Cortisol in symptomatic HIV stage 11 participants on ART.

The study finally concludes that HIV infection has been observed to increase malaria infection in symptomatic HIV stage 11 participants on ART and antiretroviral therapy had some stabilizing effect and immune recovery on symptomatic HIV participants on ART. Also, HIV infection was observed to cause a stimulatory effect on the endocrine glands to release LH, FSH, Progesterone and Estrogen and an inhibitory effect on the release of Testosterone from the gonads.

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