Original Research Article

Evaluation of serum sex hormones and CD$_4^+$ count among HIV patients on HAART, HAART naïve patients and apparently healthy subjects in Sokoto, Nigeria

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ABSTRACT

Background: Acquired Immunodeficiency Syndrome (AIDS) is a chronic disease associated with Human Immunodeficiency Virus (HIV) which progressively induces depletion of CD$_4^+$ T cells, and increased vulnerability to opportunistic infections. Previous reported studies associated HIV-infected men with sexual dysfunction; hypogonadism is the most common endocrinological disorders. Its prevalence remains poorly defined and widely ranging from different studies.

Methods: This study evaluated a total of 135 serum sex hormones (testosterone, estrogen, follicle stimulating hormone and luteinizing hormone) and its correlation with CD$_4^+$ counts among HIV patients on HAART, HAART naïve patients and negative control subjects (n=45). CD$_4^+$ cell counts were estimated using standard flow cytometry method and serum sex hormones by competitive enzyme immunoassay technique.

Results: There were significantly lower testosterone and CD$_4^+$ levels (p<0.05) among HIV positive HAART naïve men compared to negative control. LH and FSH indicated significant increased (p<0.05) among HIV positive men on HAART.

Conclusions: Antiretroviral therapy improves sexual functions in males infected with human immunodeficiency virus. Hence, further study to evaluate its effects on other sexual behaviors.

Keywords: Acquired Immunodeficiency syndrome, Antiretroviral therapy, Antiretroviral naïve patients, Sex hormones

INTRODUCTION

Nigeria has the second largest Human Immunodeficiency Virus (HIV) epidemic in the world. In accordance with it population size, 1.9 million Nigerians were living with HIV in 2018. HIV belongs to the family of retrovirus that causes Acquired Immunodeficiency syndrome (AIDS). A chronic viral disease associated with the progressively induced depletion of CD$_4^+$ T cells and increased vulnerability to opportunistic infections. HIV
infection is associated with functional derangement of virtually every endocrine system of human body. Functional derangement of sex hormones may result either from primary testicular failure or inadequate signaling from the pituitary or hypothalamus. High prevalence of hypo-gonadism was reported in several studies conducted in HIV infected males during pre-antiretroviral therapy.

Highly active antiretroviral therapy (HAART) has been extremely successful in suppressing HIV infection restoring immune function. However, HAART has also been implicated to cause sexual dysfunction indirectly affecting people living with HIV/AIDS (PLWHAs) and their inability to adhere to antiretroviral therapy. Sexuality is an intrinsic part of person's wellbeing, knowledge gaps exist on our understanding of issues of sexuality outside the risky behaviors paradigm among PLWHA on HAART in Sokoto and Nigeria at large. Very few studies have been conducted in Nigeria on the prevalence of hypogonadism in HIV infected males. This research would explore sex profile hormones among HIV-infected men in Nigeria is poorly defined and evaluate it correlation to CD4 count.

METHODS

This study was conducted from 3rd July, 2017 to March, 2018 at the Specialist Hospital Sokoto, Nigeria. Sokoto State is located at the extreme part of North-Western Nigeria between longitude 3 and 7o East and latitude 10 and 14o North of the equator. Sokoto shares borders with Niger Republic to the North, Kebbi State to the South, Western and Zamfara State to the east. The state covers a total land area of about 32,000 square kilometers and a population of 4,602298 million based on 2013 projection. Sokoto state has semi-arid climate and vegetation is largely Sudan Savannah with an annual rainfall of between 500 and 1300 mm and temperature range between 15 and over 400C during warm days.

Inclusion criteria

- HIV seropositive male aged 15-60 years presented with no clinical conditions likely to affect serum concentrations of sex hormones
- Apparently healthy male subjects as negative controls.

Exclusion criteria

- HIV-positive patients with history of concomitant comorbidities such as diabetes mellitus, chronic kidney disease (serum creatinine > 1.5 mg%), chronic liver disease, past history of meningitis, stroke, cryptococcal infection and other related conditions.
- HIV-positive patients with established cases of sexual dysfunction and/or infertility before commencement of HAART therapy.

- HIV-positive patients with substance abuse opiates (including heroin and methadone) or marijuana
- HIV-positive patients who declined to give consent for inclusion.

A total of 135 male subjects aged 15-60 years were recruited for this study. These consisted of 45 HIV patients on HAART, 45 HAART-naive HIV patients attending the ART Clinic in Specialist Hospital, Sokoto, Nigeria and 45 apparently healthy individuals (negative controls).

Ethical approval with registration number: SHS/SUB133/VOL.1 was obtained from the Ethical and Research Committee of Specialist Hospital Sokoto, Nigeria.

The sample size for the study was calculated using the formula below

\[ n = \frac{z^2pq}{d^2} \]

Where,

- \( n \) = the desired sample when the population is greater than 10,000.
- \( Z \) = the desired normal deviate, usually set at 1.96 which corresponds to the 95% confidence level.
- \( P \) = the current prevalence rate of HIV in Sokoto which is 5.6%.
- \( q \) = 1 - \( P \)
- \( d \) = degree of accuracy desired, usually set at 0.05.

The calculated sample size was 81. However, 10% (=9 patients) were added as attrition rate. Therefore, the final calculated sample size was 90.

Experimental design

A simple random sampling technique was used to recruit 45 HIV infected patients on HAART and 45 HIV infected patients attending Antiretroviral Therapy (ART) Clinic of Specialist Hospital Sokoto that were yet to commence HAART and 45 Negative Control Subjects were also selected from staff of Specialist Hospital Sokoto (Table 1).

Sample collection

About 5ml of venous blood sample was collected at the clinic using a sterile disposable syringe and needle. 4ml of the blood was transferred into plain tubes and allowed to clot at room temperature and then centrifuged at 4000 rpm for 5 minutes. The sera were harvested and placed into other plain tubes, stored at -200C until the time of
analysis. The remaining 1 ml was transferred into a sterile EDTA specimen bottle and used for the estimation of CD4+ count within 3 hours of the blood collection.

Table 1: A cross sectional descriptive design of experimental groups and descriptions (n = 45).

| Groups    | Description of experimental groups             |
|-----------|-----------------------------------------------|
| Group I   | HIV infected patients on HAART                |
| Group II  | HAART-naïve HIV patients                      |
| Group III | Apparently healthy subjects (negative control) |

Laboratory analysis

Partec, Germany flow cytometer was used to obtain CD4 T cell count. Free testosterone, estrogen, luteinizing hormone and follicle stimulating hormone were estimated using method of competitive enzyme immunoassay technique as described by.

Principles of flow cytometer

Flow cytometer was used to obtain CD4 T cell count. In flow cytometry, cells are separated in aqueous suspension and stained with fluorescent dyes. Cells in flow cuvette are individually illuminated by excitation light source of the laser (488nm). This excitation causes dye molecules to fluorescence at characteristic color of emission. The fluorescent signals are then displayed and analyzed in histograms.

Principles of enzyme immunoassay (determination of sex hormone profile)

Serum sex hormone was carried out using standard method of estimation of testosterone, estrogen, serum luteinizing hormone, and follicle stimulating hormone.

Statistical analysis

The data generated were analyzed using Statistical Package for Social Sciences (SPSS) version 22.0. Serum sex hormones were analyzed and expressed as Mean±SEM. The results obtained were compared between different groups using ANOVA. A p-value of p < 0.05 was considered significant.

RESULTS

Majority of the HIV infected men (66.7%) were married while 25.2% were unmarried (Table 2). Among the tribes (Hausa, Fulani, Igbo and Yoruba), Hausa (73.3%) constitute the largest population of HIV infected men and Fulani (3.7%) the least in Sokoto metropolis (Table 2).

Table 2: Demographic characteristics of the study population.

| Characteristics | n   | Percentage (%) |
|-----------------|-----|----------------|
| Marital status  |     |                |
| Married         | 90  | 66.7           |
| Single          | 34  | 25.2           |
| Widowed         | 9   | 6.6            |
| Divorce         | 2   | 1.5            |
| Total           | 135 | 100            |
| Ethnicity       |     |                |
| Hausa           | 99  | 73.3           |
| Fulani          | 5   | 3.7            |
| Yoruba          | 11  | 8.1            |
| Igbo            | 14  | 10.5           |
| Others          | 6   | 4.4            |
| Total           | 135 | 100            |

Table 3: Age (years) and gender distribution of HIV-positive on HAART, HIV-positive, HAART-naive and HIV-negative controls.

| Group   | Group I | Group II | Group III | Total number of groups |
|---------|---------|----------|-----------|------------------------|
| Age     | Male | Male | Male |                         |
| group(years) |     |       |      |                        |
| 15-24   | 12   | 5     | 2    | 39                     |
| 25-34   | 5    | 4     | 7    | 41                     |
| 35-44   | 5    | 5     | 8    | 32                     |
| 45-54   | 1    | 6     | 5    | 17                     |
| 55-64   | 0    | 3     | 1    | 6                      |
| Total   | 23   | 23    | 23   | 135                    |

Group I= HIV on HAART, GROUP II= HIV-positive HAART-naive, Group III= controls, HAART= highly active antiretroviral therapy, HIV= Human immune virus.

Table 4: Comparison of serum sex hormones and CD4+ count among male subjects.

| Parameters       | Group I               | Group II              | Group III              |
|------------------|-----------------------|-----------------------|------------------------|
| Testosterone (ng/ml) | 3.40±0.70*           | 3.43±0.59*           | 10.34±0.56*          |
| Oestrogen (pg/ml)   | 83.84±7.82           | 104.15±10.32         | 102.32±6.25          |
| LH (MIU/ml)        | 10.79±2.93*          | 7.03±0.88*           | 3.94±0.54            |
| FSH (MIU/ml)       | 12.05±2.86*          | 5.51±1.00            | 4.05±0.78            |
| CD4+ (cell/mm3)    | 280.63±42.41*        | 245.79±45.48*        | 790.32±36.50*        |

Values are mean ± SEM, n= number of subjects, the values bearing asterisk differ significantly with the respective control at p <0.05 (*), using ANOVA. Group I= HIV-positive on HAART, Group II= HIV-positive HAART-naive and Group III= HIV-negative control.
Figure 1: Comparison of serum sex hormone among male HIV on HAART, HIV HAART-Naïve and controls.

Table 5: Correlation of CD4+ count with sex hormones among HIV positive men on HAART, HAART-naïve and HIV negative controls.

| Parameters | r-value | p-value | Remark |
|------------|---------|---------|--------|
| Testosterone (ng/ml) | 0.682** | <0.001 | SS     |
| Oestrogen (pg/ml) | 0.025 | 0.140 | NSS    |
| LH (MIU/ml) | -0.181 | 0.025 | SS     |
| FSH (MIU/ml) | -0.271* | 0.027 | SS     |

**=correlation is significant at 0.01 level (2-tailed), LH= Luteinizing hormone, FSH= Follicular stimulating hormone.

DISCUSSION

This study indicated a significant decrease in serum levels testosterone in HIV infected male on HAART and HAART naïve male compared with negative control (p<0.05). This finding is in agreement with previous reports. Hypogonadism is common among HIV infected male with incidence between 29-50% without HAART and 20-30% on HAART.

There were significant increase in sex hormone profile levels of FSH, LH and estrogen among HIV positive and HAART naïve males compared with negative control. These significant differences might be due to the relationship between LH, FSH, estrogen and the testosterone. LH and FSH regulate the secretion and release of testosterone.

The finding also shows significant decreased levels of CD4+ count among HIV on HAART and HAART naïve in male (p < 0.05). This is in agreement with previous report. HIV infection and its stages of progression decreased CD4+ count and have been reported to be associated with changes in serum sex hormones level.

CONCLUSION

HIV is associated with changes in sex hormones which may lead to sexual dysfunction in infected individuals and probably antiretroviral therapy may improve sexual functions. Further study is needed to evaluate its effect on other sexual behavior.

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