SEX HORMONES CHANGES ASSOCIATED WITH MENSTRUAL CYCLE IN HIV INFECTED FEMALES AT NAUTH, NNEWI, SOUTHEAST NIGERIA.

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Abstract

Background: The association of Human Immune deficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) and sex hormone changes may grossly affect the reproductive health in affected women. This was a prospective study done at Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria to evaluate the sex hormone changes in HIV infected women of reproductive age group during menstrual cycle. **Materials and methods**: A total of 90 women (60 HIV

positive, 30 Control) were studied. After detailed medical examination, a well-structured questionnaire was self-administered. Blood samples were collected under sterile conditions during the follicular and luteal phases of menstrual cycle after due informed consent had been sought and obtained. The samples were analyzed for sex hormones (Progesterone, estradiol and testosterone) using Enzyme Linked Immunosorbent Assay (ELISA) method. **Results**: The result showed that the sex hormones (Progesterone, estradiol and testosterone) were significantly lower at both phases of the menstrual cycle in HIV infected women when compared to the Control ($P<0.05$). **Conclusion:** The study revealed some degree of hypogonadism in HIV infected women which may have some implication in their reproductive life.

Keywords: Sex hormones, HIV Women, Menstrual Cycle, Southeast Nigeria

Introduction

The Human Immune deficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) is a leading cause of morbidity and mortality among women of reproductive age in Nigeria (UNAIDS 2011; NACA 2012). 1.72 million (58%) out of 3.1 million people living with HIV/AIDS in Nigeria are females within their reproductive age (15-45 years) (UNAIDS 2011; UNAIDS 2012). This shows increased burden of HIV on the female population when compared to their male counterparts.

HIV has been known to impact negatively on women's reproductive health (Ikechebelu *et al*., 2002; Fallahian and Ilkhani, 2006). The negative reproductive impact ranges from menstrual disorders to outright infertility. Some of these disorders may be as a result of deranged ovarian function with far reaching effects on sex hormones.

The present study was therefore designed to evaluate sex hormones changes associated with menstrual cycle in HIV infected premenopausal female subjects in Nnewi, Anambra State, Nigeria.

Materials and methods

The sample population consisted of ninety premenopausal female participants within the age range (15-45years). Thirty participants were apparently healthy controls recruited amongst the Hospital staff. Thirty participants were HIV seropositive females who have not been placed on antiretroviral therapy while the remaining thirty were HIV seropositive female participants who have been placed on antiretroviral therapy for not less than six months. They were recruited at the HIV clinic of Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State Nigeria.

After obtaining informed consent of the participants, a well detailed questionnaire was administered to each participant to ascertain their medical and reproductive history. 8 millimeters of venous blood was collected aseptically between 9.00am and 12noon from each participant at follicular ($7\text{-}13^{th}$ day) and luteal ($21\text{-}23^{rd}$ day) phases of menstrual cycle. All the participants were double screened for malaria parasite and HIV infection using rapid antigen diagnostic techniques for *malaria falciparum* and immunoassay and immunochromatographic methods for HIV screening respectively. The serum content was separated immediately after clot retraction, labeled and stored at $-20^{o}C$ for determination of estradiol, progesterone and testosterone using ELISA (Enzyme Linked Immunosorbent Assay) method.

The ethics committee of NAUTH Nnewi approved the study design and only those who gave their consent were recruited for the study.

Inclusion and Exclusion criteria: only the participant adjudged as HIV stage 2 were recruited for the study. HIV stage 1, stage 3 and stage 4 were excluded, participant with malaria parasite infection as at the time of study were excluded, participants on contraceptives were excluded, women with previous history of infertility prior to the study and participants who were co-infected with tuberculosis were also excluded from the study. Hence the female participants used were those with no prior fertility problems until the existence of HIV infection.

Methods

Antibodies to HIV-1 and HIV-2 in Human Plasma were detected using Abbott Deterimine system, Immunoassay method [(Trinity Biotech UniGold Assay Kit (Trinity Biotech PLC, Ireland)] and imunochromatographic method [(HIV 1 and 2 STAT-PAK Assay kit (Chembio diagnostic system, INC New York, USA)] respectively.

Determination of Progesterone, Estradiol and testosterone were done using Enzyme Linked Immunosorbent Assay (ELISA) kits (Glory Science Laboratory USA)

Statistical analysis

The version 16 of SPSS package was used in statistical analysis. The variables were expressed as mean (±SD). The student t-test and analysis of variance (ANOVA) and post-hoc (LSD) were used to assess significant mean differences. Graph Pad Prism version 5.03 was used for graph presentations.

Results

Levels of Sex Hormone (Progesterone) at follicular and luteal phases of menstrual cycle

The mean (±SD) serum Progesterone concentration (ng/ml) in HIV seropositive females and HIV seropositive females on ART was not significantly different between follicular (1.9±0.7, 2.5±2.0) and luteal (1.9±0.3, 2.1±1.8) phases of menstrual cycle (P>0.05 respectively). But the mean serum progesterone concentration (ng/ml) dropped significantly at follicular phase (4.4±2.5) compared with the luteal phase (8.7±4.9) of menstrual cycle in Control female subjects (P<0.05).

When the mean progesterone concentration (ng/ml) at follicular and luteal phases of menstrual cycle were compared between the Control group and the Test groups, the mean progesterone concentration dropped significantly in HIV seropositive (1.9±0.7, 1.9±0.3) and HIV seropositive on ART (2.5±2.0, 2.1±1.8) compared with follicular and luteal values in the Control female subjects (4.4±2.5, 8.7±4.9) (P<0.05 respectively) (See Fig 1).

Levels of Sex Hormone (Estradiol) at follicular and luteal phases of menstrual cycle

The mean (±SD) serum estradiol Concentration (pg/ml) in HIV seropositive females and HIV seropositive females on ART was not significantly different between follicular (24.2±18.7, 32.8±20.2) and luteal (18.7±11.2, 38.4±24.0) phases of menstrual cycle (P>0.05 respectively). Conversely, the mean estradiol value (pg/ml) dropped significantly at follicular phase (80.9±36.6) compared with luteal phase (94.1±50.1) of menstrual cycle in Control female subjects (P<0.05).

When the mean estradiol concentration (ng/ml) at follicular and luteal phases of menstrual cycle were compared between the Control group and Test groups, the mean estradiol dropped significantly in HIV seropositive females (24.2±18.3, 18.7±11.2) and HIV seropositive females on ART (32.8±20.2, 38.4±24.0) compared with follicular and luteal values in the Control female subjects (80.9±36.6, 94.1±50.1) (P<0.05 respectively) (see fig 2).

Levels of Sex Hormone (Testosterone) at follicular and luteal phases of menstrual cycle

The mean (±SD) serum testosterone level (ng/ml) in HIV seropositive females was not significantly different between follicular (0.30±0.17) and luteal (0.30±0.15) phases of the cycle (P>0.05). In HIV seropositive females on ART, there was no significant difference in the mean testosterone concentration (ng/ml) between the follicular (0.41±0.27) and luteal (0.36±0.15) phases of menstrual cycle (P>0.05). But the mean serum

testosterone value (ng/ml) dropped significantly at luteal (0.81±0.27) phase of menstrual cycle compared with follicular phase (1.08±0.48) in the Control female subjects (P<0.05).

When the Mean Testosterone concentration (ng/ml) at follicular and luteal phase of menstrual cycle were compared between the Control group and Test groups, the Mean Testosterone level dropped significantly in HIV seropositive females (0.30±0.17, 0.30±0.15) and HIV seropositive females on ART (0.41±0.27, 0.36±0.15) compared with the corresponding values in the Control female subjects (1.08±0.48, 0.81±0.27) (P<0.05 respectively).

The pos hoc analysis showed significantly higher mean testosterone concentration (pg/ml) at follicular and luteal phases of menstrual cycle in HIV seropositive females on ART (0.41±0.27, 0.36±0.15) compared with the corresponding values in HIV seropositive female subjects (0.30±0.17, 0.30±0.15 respectively) (See Fig 3).

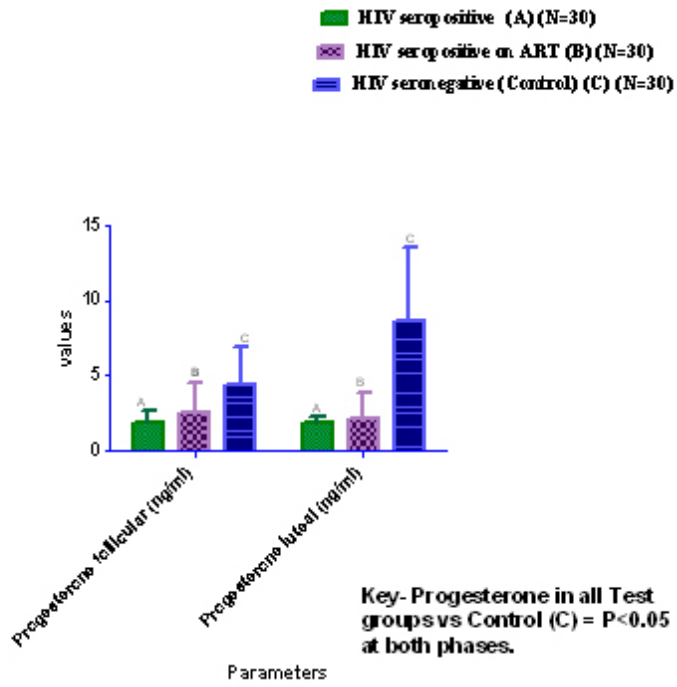
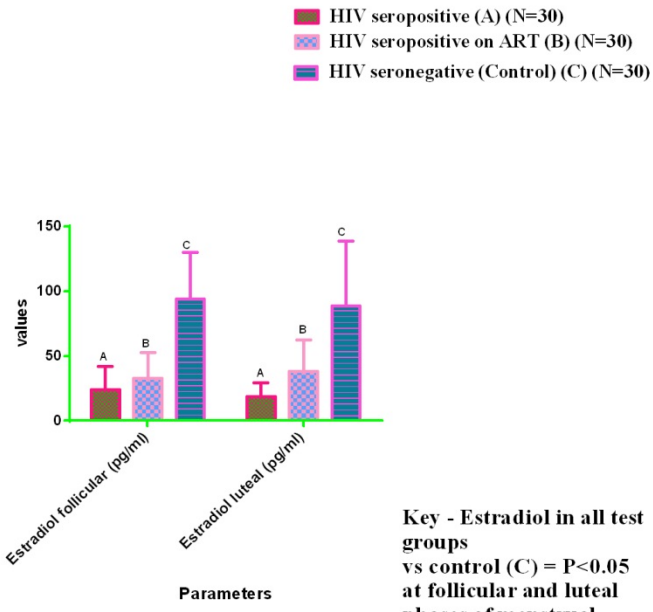
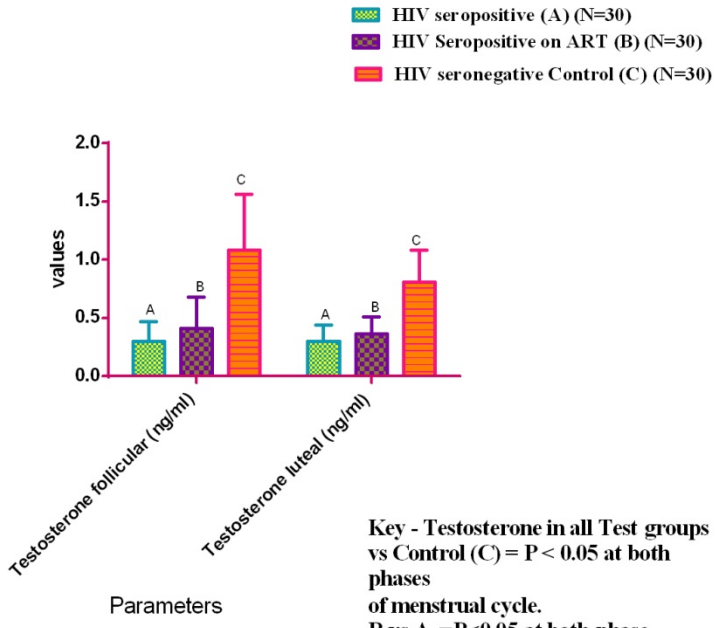


Fig 1: Comparison of mean (±SD) serum levels of Progesterone in Test groups and Control group at Follicular and luteal phases of menstrual cycle



Key - Estradiol in all test groups vs control (C) = P<0.05 at follicular and luteal phases of menstrual cycle.

Fig 2: Comparison of mean (±SD) serum levels of Estradiol in Test groups and Control group at Follicular and luteal phases of menstrual cycle



Key - Testosterone in all Test groups vs Control (C) = P < 0.05 at both phases of menstrual cycle.
B vs A =P<0.05 at both phase of menstrual cycle.

Fig 3: Comparison of mean (±SD) serum levels of Testosterone in Test groups and Control group at Follicular and luteal phases of menstrual cycle

Discussion

The present study showed that the mean serum levels of progesterone and estradiol in HIV seropositive females was not significantly different between follicular and luteal phases of menstrual cycle. This contrasts the observation in apparently healthy subjects where differences in hormonal levels exist between the two phases of the menstrual cycle. FSH and LH are usually higher at the follicular phase and peak at the mid cycle to enable ovulation take place (Norman, 2001) while Progesterone and estradiol are usually higher at the luteal phase. The absence of this normal physiological balance in the test subjects may cause changes in the cycle which may affect reproductive function. Significant reductions in the level of progesterone at the luteal phase may disturb the sustenance of pregnancy and lead to spontaneous abortion while gross reductions in these hormones (hypogonadism) may cause failure of menstruation or significant abnormality in menstrual cycle. All these factors may play significant role in the pathogenesis of infertility in affected women.

The serum levels of progesterone and estradiol levels were significantly lower in HIV seropositive and HIV seropositive females on ART at both follicular and luteal phases of menstrual cycle. The implication of grossly reduced progesterone is primary ovarian failure. This means that the anterior pituitary may be overworking itself to stimulate a poorly responding ovary. The abnormally low progesterone signifies hypogonadism and may lead to menstrual and reproductive failure. Some previous studies done developed countries (Merrill *et al*., 1989; Tracy and Cerami, 1990) produced a similar report. Hypogonadism is a significant cause of infertility in HIV women (Grinspoon e*t al*., 1997).

The appreciation in the level of progesterone in HIV seropositive subjects on ART suggests stimulatory effects of the treatment on the gonads with intact negative feedback mechanism thereby resulting in the restoration of the gonadal functions showing some beneficial effects and a tendency to return to normal. This may reduce the incidence of menstrual abnormality and infertility. Unfortunately however, studies have reported that prolonged use of ART especially protease inhibitors have been associated with other problems such as hyper prolactinaemia (Hutchinson *et al*., 2000). Hyper prolactinaemia has been associated with increased incidence of anovulation and hence infertility (Ikechebelu *et al*., 2002).

Studies of ovarian function in HIV infected women have produced controversial reports. Whereas some authors (Chargwin *et al*., 1996; Harlow *et al*., 2000) reported significant effects of HIV on ovarian function, others (Shah *et al*., 1994; Ellerbrock *et al*., 1996) reported no significant change in ovarian function. This controversy is believed to be due to the fact that most of the studies were done in advanced or industrialized countries where

women who were advanced in age were the participants and the studies did not consider the phases of their menstrual cycle. Such women were said to have poor ovarian reserve since most of them were premenopausal (Panda *et al*., 2013). In the present study however, the participants were women within their reproductive age (15-45years) at different phases of their menstrual cycle and the effects of HIV on the ovarian function will be more pronounced.

The significantly lower level of testosterone observed in HIV seropositive and HIV seropositive female subjects on ART when compared to Control females is also due to the reduced ovarian function. In normal physiology, testosterone is produced by the ovaries in the females in very small quantities which serve to maintain muscle mass and hence prevents weight loss. In diseased individuals, this quantity becomes much smaller because of reduced ovarian function and is not able to achieve its physiological function anymore hence the excess weight loss that is associated with these diseases (HIV inclusive) (Huang *et al*., 2003).

In Conclusion, the study showed significant reduction in the sex steroid hormones indicating reduced ovarian function (hypogonadism) which accounts for menstrual changes that can lead to infertility in the affected women.

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