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IMPACT OF HIV ON FERTILITY IN NAÏVE PREMENOPAUSAL FEMALES

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Abstract

This study investigates the fertility status of naïve HIV-infected females of childbearing age. Approved by the Medical Ethical Committee of the University of Nigeria Teaching Hospital (UNTH), Enugu, and with informed consent obtained from all participants, the study involved 80 HIV-positive females aged 20 to 35 years from the PEPFAR HIV clinic at UNTH, Enugu. A control group of 80 age-matched, HIV-negative females was also included. Blood samples were collected and analyzed using standard methods. HIV diagnosis was confirmed through the WHO HIV test algorithm. Flow cytometry was utilized for CD4+ T lymphocyte enumeration, while hormone levels were assessed using competitive ELISA. Results indicated that serum oestradiol and progesterone levels in HIV-positive patients were significantly lower ($p < 0.001$) compared to the control group. However, in patients with CD4 counts ≥ 500 cells/µl, hormone levels did not significantly differ from controls ($p > 0.05$). Additionally, the CD4 count in HIV-positive patients was significantly reduced compared to the control group ($p < 0.001$). Intra-batch variations for oestradiol and progesterone were 0.53% and 1.30%, respectively, while inter-batch variations were 1.40% and 6.8%. These findings suggest that while HIV infection is associated with lower hormone levels, high CD4 counts may mitigate some effects on fertility.

INTRODUCTION

Oestrogens are hormones responsible for the development and maintenance of the female sex organs and female secondary sex characteristics. Oestrogens affect calcium homeostasis and have a beneficial effect on bone mass. They decrease resorption, and in pre- pubertal girls, accelerate linear bone growth and results in epiphyseal closure. The most compelling explanation for the suppressive effects of estrogens on bone resorption, at least in cancellous bone, is that estrogens promote osteoclast apoptosis (Almeida et al., 2017). It has also been reported

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that oestrogens play a principal role in the skeletal growth and bone homeostasis. In women, oestrogen deficiency after menopause accelerates frequently osteoclastic bone resorption. Oestrogen depletion is associated with loss of bone mineral content, an increase in stress fracture, post-menopausal osteoporosis and increases in production of cytokines, such as interleukin-1, interleukin-6 and tumour necrosis such as interleukin-1, interleukin-6 and tumour necrosis factor-alpha (Okman-Kilic, 2015). More than 20 oestrogens have been identified but only 17 β-Oestradiol (denoted by E2) and oestriol (also denoted by E3) are routinely measured clinically. The most potent oestrogen, secreted by the ovary is oestradiol and because it is derived almost exclusively from the ovaries, its measurement is often considered sufficient to evaluate ovarian function. It has been reported that high-dose estrogens, cause fusion of the growth plates and prevent undesirable longitudinal growth in girls. Low-dose estrogens in turn can trigger the pubertal growth spurt in girls with Turner syndrome or constitutional delay of growth and puberty (CDGP), without adverse effects on ultimate height (Ranke, 2013). It has been demonstrated that oestrogens can promote cell proliferation and increase extracellular matrix expression of axial and perpendicular growth plate chondrocytes during postnatal development, especially type II collagen expression (Shi et al., 2017).

Progesterone is generated in the ovary, the adrenal gland, the placenta during pregnancy and the nervous system, in which it plays an important role as a neurosteroid. This steroid is the principal intermediate for circulating androgens and estrogens. Progesterone like oestrogens, a female sex hormone belongs to a class of hormones called progestogens and it is the major naturally occurring human progestogen (Zubeldia- Brenner et al., 2016). Progesterone allows the endometrial transition from a proliferative to the secretory stage and facilitates blastocyst nesting. These characteristics explain the ethymology of the hormone′s name, which comes from the Latin "progestationem" and it is especially important in preparing the uterus for the implantation of the blastocyst and in maintaining pregnancy (Taraborrelli, 2015). In nonpregnant women, progesterone is secreted mainly by the corpus luteum while during pregnancy, the placenta becomes the major source of this hormone (Coomarasamy et al., 2019). Progesterone and progesterone receptors (PR) are essential for the development and cyclical regulation of hormone-responsive tissues including the breast and reproductive tract. It has also been reported that altered functions of PR isoforms contribute to the pathogenesis of tumors that arise in these tissues. In the breast, progesterone acts in concert with estrogen to promote proliferative and pro-survival gene programs. They also participate in the regulation of the menstrual cycle, breast and uterine growth and in the maintenance of pregnancy (Haymond and Gronowski, 2006; Diep et al., 2015).

HIV is primarily a sexually transmitted disease. Homosexual, bisexuals and heterosexual transmissions all can occur. Although sexual intercourse between the males has remained the greatest risk for transmission in developed nations of western Europe and the United States. Same study posited that heterosexual transmission is increasing in those regions but still remains less common than in Africa, Asia, or parts of the Caribbean (Beyrer et al., 2012). Worldwide, sexual transmission accounts for the majority of cases of HIV infection (Daar and Corado, 2016). Another study suggested that men who have sex with men (MSM) who were married were more likely to be infected with HIV/AIDS implying that their spouses also had greater risk of HIV infection (Qi et al, 2015). The important facts that promote heterosexual transmission include more sexual partners, frequent change of sexual partners, unprotected intercourse, presence of additional sexually transmitted diseases, lack of male circumcision, social vulnerability of women and young persons and economic and political instability of the country/community. Among these factors, multiple sex partners was defined as having more than one sexual partners over a period of time. These can be either serialized partners; one after the other, or simultaneous or concurrent; different sexual partners that overlap in time (Mutinta, 2014). Other sources of HIV infection include parenteral infection and mother to child transmission. Availability of highly active antiretroviral therapy

(HAART) has really saved mankind from the dreaded scourge of HIV/AIDS. HAART has actually transformed the very short life expectancy in people living with HIV to a near normal life expectancy. However, HIV is associated with deadly osteopenia and osteoporosis with people living with HIV experiencing these anomalies earlier in life than their HIVnegative counterparts (Finnerty et al., 2017). This study is aimed at evaluating the fertility status of naive human immunodeficiency virus/acquired immune deficiency syndrome-infected premenopausal females.

MATERIALS AND METHODS

Study population

Two categories of subjects were used. The first category was 80 HIV positive females aged between 20 to 25 years at the President's Emergency Action Plan for AIDS relief (PEPFAR), HIV Clinic, University of Nigeria Teaching Hospital, Enugu. The second category of subjects was the control group of 80 apparently healthy and HIV negative age-matched female volunteers.

A formal approval for this work was obtained from the Medical Ethical Committee of the University of Nigeria Teaching Hospital, Ituku Ozalla, Enugu. Informed consent was obtained from the patients prior to their inclusion in this study. These HIV infected females were rescreened and confirmed positive. They were counselled one on one as data on their demographic characteristic and behavioural factors were being obtained. Pregnant women and breast feeding mothers were excluded from the study. Similarly, patients who were on antiretroviral therapy prior to this study were not selected. The HIV/AIDS patients were divided into three groups according to Centre for Disease Control and Prevention Criteria (CDC) system (Selik et al, 2014). This system is based on three ranges of CD4+ counts viz: (1) ≥500, (2) 499-200 and (3) <200 cells/µl.

Collection of samples

All the venous blood samples (5 ml) were collected from the medial cubital vein with minimal venous stasis, dispensed into plain tubes, allowed to clot and the sera separated by centrifugation at 3,000 g for 10 min. The sera were used for HIV serology and hormone analyses. Where the analyses were not possible the same day, the samples were stored at 4°C till the next day for analyses. HIV re- screening and confirmation tests were carried out using two enzyme linked immunosorbent assay rapid screening kits based on WHO] system two, serial algorithm for detecting antibodies to HIV I and 2 (Kosack et al., 2017) and to determine it, a rapid screening kit (Alere Medical Company Ltd, Japan) and Immunocomb II (Organics, France) were used in the study. The quantitative determination of serum oestradiol and progesterone were carried out using micro plate competitive enzyme immunoassay with Accubind ELISA kit (Monobind Inc., Lake Forest, CA 92630, USA).

Method for intra-and inter-batch variations for the determination of the precision of the hormonal assays

Blood samples were collected from ten apparently healthy individuals. The samples were allowed to clot and the sera separated and pooled. The serum pool was then dispensed in aliquots of 20 × 2 ml. The first 10 aliquots were used for the assessment of intrabatch variation while the second 10 aliquots were used for the interbatch variation. The intra-batch variation was carried out as a batch while the inter-batch variation was carried out by inserting one aliquot in a batch of analysis until the 10 aliquot replicates were determined both for oestradiol and progesterone.

Statistical analysis

The results were expressed as Mean ± Standard deviation (M ± SD). The data were analyzed using graph pad Instat version 3.01, Graph pad software, San Diego, California, USA, while the test of significance was based on probability $p < 0.05$. Comparisons between groups were performed using One-way-ANOVA, Bonferroni's multiple comparison test and student t-tests depending on the number of variables.

RESULTS

Demographic parameters of the test subjects

Table 1 shows the mean age (years) 29.1 ± 3.52, 29.25 ± 2.79, 29.51 ± 3.61 and 28.35 ± 3.5 for the overall test subjects, test subjects with CD4+ (cells/µl) ≥ 500, 499 - 200 and <200, respectively. On comparison of the control value (29.78 ± 3.18) with the patients and their subgroups based on CD4+ classification showed no significant difference ($p > 0.05$). The table also showed the breakdown of the 80 HIV patients based on the type of HIV they have as follows: HIV-1 (-53 patients), HIV-2 (-7 patients) and HIV-1 and HIV-2 (-20 patients). Different HIV types were further sub-divided by the CDC HIV staging depending on the levels of CD4+. The result of the patients also showed massive proteinuria with the declining CD4+ count. However, the 6.4% proteinuria found in the control group may be as a result of latent sicknesses not captured in the course of this study since the controls were only apparently healthy.

Mean Age (years)	29.78 ± 3.18	29.1 ± 3.52[aa]	29.25 ± 2.79[aa]	29.51 ± 3.61[aa]	28.35 ± 3.5[aa]
HIV - 1 (n)	-	53	10	26	17
HIV -2 (n)	-	7	2	3	2
HIV 1 and 2 (n)	-	20	8	8	4
Proteinuria (%)	6.4	47	35	45	69

[aa]represents $P > 0.05$.

Precision of the hormone assay

The intra-batch variation for serum oestradiol and progesterone were 0.53 and 1.30%, respectively while their respective inter-batch variations were 1.40 and 6.8% as shown in Table 2.

Hormonal assays

Analysis of serum oestradiol showed a significant difference ($p < 0.05$) between the test subjects and the control. As shown in Table 3, the mean serum oestradiol levels (pg/ml) were 134.01 ± 64.39, 208.30 ± 9.92, 152.16 ± 7.03, 40.0 ± 10.29 and 222.69 ± 75.14, for the overall test subjects, test subjects with CD4+ (cells/µl) ≥ 500, 499 - 200, <200 and control, respectively. The mean serum progesterone (ng/ml) were 8.59 ± 4.34, 15.16 ± 1.50, 8.44 ± 1.08, 3.49 ± 1.48 and 16.06 ± 5.94, respectively for total test subjects, test subjects with CD4+ (cells/µl) ≥500, 499 - 200, <200 and control, respectively. Comparison of the control and test groups showed a significant decrease ($p < 0.05$). The mean blood CD4+-lymphocyte count of the subjects in Table 3 was 359.39 ± 158.63, 552.0 ± 26.38, 384.54 ± 74.40 and 151.61 ± 38.87 cells/µl for overall patients, patients with CD4+ count ≥500, 499-200 and <200 cells/µl, respectively while that of the control was 787.09 ± 106.18 cells/µl. There was a significant difference between the control group and various groups of the subjects ($P < 0.05$).

Table 2. Precision of the hormonal assays

S/N		MEAN ± SD	C.V. (%)	N
1	Serum oestradiol (pg/ml)	(a) Intra-batch variation 218.6 ± 2.04	0.53	10
		(b) Inter-batch variation 206.72 ± 3.62	1.40	10
2	Serum progesterone (ng/ml)	Intra-batch variation 15.4 ± 0.60	1.30	10
		Inter-batch variation 14.2 ± 0.74	6.8	10

Table 3. Fertility status of patients and HIV progression

Parameters	Controls		Test subjects		CD4 counts (cells / µl)		
				≥ 500	499 – 200	< 200	
Serum oestradiol (pg/ml)	222.69 ± 75.14		134.01 ± 64.39**	208.30 ± 9.92#	152.16 ± 7.03**	40.0 ± 10.29**	
Serum progesterone (ng/ml)	16.06 ±5.94			15.16 ± 1.50#	8.44 ± 1.08**	3.49 ± 1.48 **	
CD_4^+ count (cells / µl)	787.09 ± 106.18	8.59 ± 4.34** –	359.39 ± 158.63**	552.0 ± 26.38**	384.54 ± 74.40**	151.61 ± 38.87**	
	N = 80		N = 80	N = 20	N = 37	23	

Mean ** Indicates significant difference P < 0.05 while #Indicates non – significant difference P > 0.05 using Bonferroni's multiple comparison test

DISCUSSION

The various stages of HIV-infection, asymptomatic stage inclusive, are closely associated with quantifiable medical laboratory findings and early detection is important due to the high risk of transmission that precedes seroconversion and also because it provides an opportunity to improve health outcomes with an early antiretroviral therapy (Tüzüner et al., 2016).

Surrogate markers of HIV infection are by definition, measurable traits that correlate with development of clinical AIDS. Gold standard markers for monitoring HIV-infected individuals are CD4+ T cell count and HIV viral load which are expensive tests to run (Houshang et al., 2015). In order to provide valuable information on prognostic significance in resource-poor settings, available surrogate markers have been investigated to identify and monitor patients who are at great risk of disease progression. Amongst them are haemoglobin and total lymphocyte count and have been shown to be reliable predictors of successful treatment outcome comparable to the increase in CD4+ count (Shete et al., 2020). CD38 expression has also been shown to correlate with viraemia and has been proposed as a surrogate biomarker (Njuguna et al., 2016). Nevertheless, the most characteristic feature of AIDS

is a selective depletion of the CD4+ T-helper-inducer subset of T-cells (Okoye and Picker, 2013). Other functions of these surrogate markers of clinical AIDS include formulation of the decision on timing of medical interventions, to predict outcomes and plan future health care expenditure (Ewings et al., 2014).

The result obtained from the precision of the hormonal assays, both the inter-batch and intrabatch variations showed low values of coefficient of variations. This work corroborates the work of Greenblatt et al. (2000) who in similar study reported inter-and intra assay coefficient of variation of <15 and 10%, respectively. The result of the hormonal assays showed low levels of both hormones, oestradiol and progesterone in the HIVinfected test subjects. Hypogonadism had been reported to be a common occurrence in HIVinfected women especially those with weight loss (Dutta et al., 2017). However, on the fertility status of the HIV-infected subjects based on CDC classification of HIV/AIDS, there was no significant difference between the HIV-patients with CD4+ count ≥500 cells/µl and the control group. This, therefore, is in concurrence with the work of Cu-Uvin et al. (2000) who concluded that HIV-infected women with self-reported normal menstrual cycles have normal levels of oestradiol and progesterone. On the other hand, other scholars reported 15 to 30% infertility cases in HIV-infected females. They maintained that in HIV-infected females, all parts of the females' reproductive systems may be influenced by the infectious and pathological agents which may interfere with the reproductive function and so are risk factors for infertility (Farsimadan and Motamedifar, 2020). Moreover, HIV infection has also been reported to influence endocrine glands at several levels and hence the impairment of endocrine function in such subjects. According to the study, endocrine function was reported to be altered by the direct effect of HIV viral proteins, through generation of systemic and local cytokines and the inflammatory response and also via glandular involvement with opportunistic infection and related malignancies (Mirza et al., 2018).

Conclusion

It is clear from this study that in HIV-infected women, there were no significant differences in progesterone and oestradiol levels of the patients with CD4+ ≥500 cells/µl with the matched control group until the late stage of the infection when the CD4+ count is relatively low at which stage the hormonal levels decreased drastically. One can say at this juncture that there is abnormality of Hypothalamic-Pituitary-Gonadal axis in HIV patients with CD4+<200 cells/µl, the AIDS patients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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