Assessment of Some Reproductive Hormones and Inflammatory Cytokine Levels in HIV Infected Females on Hormonal Contraceptives in Nnewi, Nigeria

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Abstract

Objective: This was a case-controlled retrospective study aimed to assess the effect of hormonal contraceptives (progesterone based) on some reproductive hormones and cytokines in HIV infected females in Nnewi, Nigeria. **Materials and Methods:** A total of 118 premenopausal females on regular menstrual cycle aged (17-49) years were recruited based on their menstrual cycle phase (follicular (7-13th) and luteal (21-23rd day) with aid of questionnaire. 58 were HIV seropositive females [29 on hormonal contraceptive (A), 29 not on hormonal contraceptive (B)], while 60 were HIV seronegative females (controls) [30 on hormonal contraceptives (C) and 30 not on hormonal contraceptive (D)]. Reproductive hormones (FSH, LH, prolactin, progesterone, estradiol) and cytokines (TNF-α, IL-2) were assayed using enzyme-linked immunosorbent assay kits (ELISA). **Results:** TNF-α was significantly increased in HIV seropositive females on contraceptives compared with their counterparts not on contraceptives and controls on/not on contraceptives at both phases of menstrual cycle (P= 0.000). IL-2 was significantly decreased in HIV seropositive females on/not on contraceptive compared with control females not on contraceptive but significantly increased in HIV seropositive on contraceptive compared with their corresponding females not on contraceptive at follicular phase of menstrual cycle (P=0.002; 0.005 respectively). Progesterone and estradiol were significantly decreased in HIV seropositive females on contraceptives compared with their corresponding females not on contraceptives and controls on/not on contraceptives at both phases of menstrual cycle (P = 0.000, 0.001 respectively). **Conclusions:** The significant elevation in TNF-α and IL-2 in HIV infected females on contraceptives indicates active inflammation which was more marked at follicular phase of menstrual cycle. The significant decrease in ovarian hormones suggests hypogonardism and may be linked to exacerbated inflammatory reaction.

***Key-words**: HIV, contraceptives, fertility hormones, cytokines.*

Introduction

HIV pandemic is a major public health challenge. It has a great impact on humans' life, especially women

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and children. [1] This will pose a global threat to human existence if left unchecked. Contraceptive need of HIV infected females if fully met will reduce the global burden of mother-to-child HIV transmission and also prevent pregnancy. [2] However, hormonal contraceptives being a synthetic preparation alter the body physiology; this may interfere with the immune response to HIV virus such as the cytokines that are vital immunomodulators. [3] The effects of these immunomodulators on the reproductive system may subsequently affect the reproductive hormones that are essential for female fertility in the long term.

According to Stringer *et al.,*[3] hormonal contraceptives modulate the immune system in such a way that may affect the immune response to HIV infection. Research has shown that HIV impact negatively on women's reproductive health. [4,5,6] These negative impact ranges from menstrual disorders to outright infertility while some of the disorders result to deranged ovarian function with damaging effects on sex hormones. [6] However, much work has been done on cytokines and reproductive hormones changes in HIV. [6, 7] This work is designed to assess the effect of contraceptive use on some of the female reproductive hormones and cytokines in HIV infected females in Nnewi, southeast Nigeria.

Materials and Methods

Study Design

This research is a case-controlled, observational and retrospective study that assessed the effects of hormonal contraceptive use on some female reproductive hormones (FSH, LH, prolactin, estradiol and progesterone) and cytokines (IL-2 and TNF-alpha) levels in HIV infected females attending Nnamdi Azikiwe University Teaching Hospital Nnewi. The information on the contraceptive use was obtained through questionnaire. The sampling technique used for this research was the purposive sampling technique.

Study Area

The study was carried out at Nnamdi Azikiwe University Teaching Hospital Nnewi. The laboratory investigation was done in the chemical pathology laboratory department of the same hospital. The hospital is the major referral tertiary health facility, established in 1988 and is located in the heart of the commercial city of Nnewi.

Inclusion and exclusion criteria

HIV infected females on HAART aged 18-49 years on/not on hormonal contraceptive (HIV stage I and II) and age matched HIV seronegative females on/not on hormonal contraceptive were included in the study. HIV stage III and IV infected females; HIV co-infected with tuberculosis, Females with diabetes and hypertension, pregnant women and females below 18 years or above 49 years was excluded from the study.

Study population

A total of 118 premenopausal female participants (18-49yrs) were recruited for this study and they were divided into four groups as follows: **Group A:** HIV infected females on hormonal contraceptives (n=29), **Group B:** HIV infected females not on hormonal contraceptive (n=29), **Group C:** HIV seronegative females on hormonal contraceptive (Control 1) (n=30) and **Group D:** HIV seronegative females not on hormonal contraceptive (Control 2) (n=30). All the HIV infected females were on HAART for a minimum period of three years as at the time of sample collection and they were on fixed regimen as follows: Tenofovir/Lamivudine/ Efavirenz (TenoLamE) (300/300/600mg) once daily, Tenofovir/Lamivudine/Dolutegravir (TenoLamD) (300/300/50mg) once daily, Lamuvudine/Zidovudine/ Nevarapine (Combo pack) (150/300/200mg) twice daily, Abacavir/Lamuvudine (600/300mg) once daily plus a NNRTI, Atazanavir/Ritonavir (300/100mg) twice daily plus a NRTI or NNRTI, Lopinavir/Ritonavir (200/50mg) twice daily plus a NRTI or NNRTI. The Nucleoside Reverse Transcriptase Inhibitors (NRTI) include: Lamivudine, Abacavir and Tenofovir while the Non- Nucleoside Reverse Transcriptase Inhibitors (NNRTI) include: Efavirenz, Nevirapine and Dolutegravir. The Protease inhibitors include: lopinavir, ritonavir, atazanavir.

The participants on hormonal contraceptive has been on it for minimum of three years before sampling, the contraceptive used include; Depo-provera (medroxyprogestrone acetate) - an injectable hormonal contraceptive containing 150 mg of progesterone given once in three months, Jadelle an implantable hormonal contraceptive containing 150 mg of progesterone (levonorgestol) which once implanted last for a period of 5 years, Implanol an implantable hormonal contraceptives containing 68 mg of progesterone (etonogestrol) which once implanted last for a period of 3 years.

Ethical Consideration

The ethical approval for this work was sought and obtained from the ethics committee of Nnamdi Azikiwe University Teaching Hospital Nnewi (NAUTH) in accordance with Helsinki declaration by the World Medical Association (WMA) on the ethical principles for medical research involving human subjects. [8] Informed consent was also obtained from the participants before sampling.

Sample collection

Approximately 5 mls of venous blood was collected aseptically through venepuncture from the participants attending the adult antiretroviral clinic, family planning clinic and prevention of mother-to-child transmission clinic, as well as members of staff of Nnamdi Azikiwe University Teaching Hospital, Nnewi, at both follicular (9-13th day) and luteal (21-23rd day) phase of menstrual cycle on a follow-up. The blood samples were collected between 10.00am-1.00pm into a plain vacutainer tube and allowed to clot, thereafter it was spun for 5 minutes at 1500 revolution per minutes (rpm) using a bench centrifuge. The serum was separated for the analysis of reproductive hormones (progesterone, estradiol, prolactin, follicle stimulating hormone, and luteinizing hormone) and cytokines (IL-2 and TNF alpha) levels. The separated sera were preserved at -80^{o}c in the retroviral laboratory of Nnamdi Azikiwe University Teaching Hospital prior to assay.

Laboratory analysis

Determination of HIV-1/2 assays was done with Determine manufactured by Alere medical company limited, Japan by the method of Piot *et al.,* [9] HIV-1/2 assay was also done using Stat Pak kit manufactured by Chembio diagnostic systems incorporated as described by Chembio, [10] while, HIV confirmation was done with Uni-Gold manufactured trinity Biotech Plc, Ireland using the method described by Klarkowski *et al*., **[11]**

Prolactin was assayed using ELISA method as described by Smith *et al*., [12] FSH and LH were assayed using ELISA method as described by Baastal *et al.,* [13] while, Progesterone and estradiol were assayed using ELISA method as described by Edward *et al*., [14]

Tumor Necrotic Factor Alpha was assayed using ELISA method as described by Hedeyati *et al*., [15] and Interleukin**-2** by ELISA method as described by Malek *et al*., [16] All the test kits used were produced by Melsin Medical Company limited, Changchun China.

Anthropometric data collection

The blood pressure was obtained using mercury sphygmomanometer. The height (meter) was recorded with the use of a meter ruler and the weight (kg) was taken using a standard weighing scale. The body mass index (BMI) kg/m^2 was obtained using the formula; weight (kg)/height (m^2)

Statistical Analysis

Statistical package for social sciences (SPSS) version 21 was used for the statistical analysis. Student t-test was used to compare two independent variables while ANOVA was used to compare more than two independent variables and the post-hoc was done using Fishers least significance difference (LSD) for group comparison to assess significant mean difference. Pearson correlation was used to correlate the different parameters. Statistical significance between test group and controls was taken at $p<0.05$.

Results

Demographic characteristics

A total of 118 premenopausal female participants were

recruited through questionnaire. 58 of the participants were HIV infected (29 on hormonal contraceptive and 29 not on hormonal contraceptive), while 60 were HIV seronegative (30 on hormonal contraceptives and 30 not on hormonal contraceptives). The study population are predominantly traders 95 (80%), few civil servants 15 (13%) and students 8 (7%). The educational levels are as follows; No formal education 9(8%), primary education 18(15%), secondary education 61(52%) and tertiary education 30(25%) (Table 1).

Levels of TNF-α (pg/ml) and Interleukin-2 (ng/ml) in HIV infected females and control females on/not on hormonal contraceptives.

The mean serum TNF-α was significantly higher in HIV infected females on/not hormonal contraceptives (8.89±4.14, 3.07±0.61) and control females on hormonal contraceptive (2.32±0.78) compared with the control females not on hormonal contraceptives (1.30±0.63) at follicular phase of menstrual cycle (p=0.000 respectively). Similarly, The mean TNF-α was significantly higher in HIV infected females on/not on hormonal contraceptives (5.93±2.48, 3.82±1.22) and control females on hormonal contraceptive (6.76±9.20) compared with control females not on hormonal contraceptives (1.78±0.36) at luteal phase of menstrual cycle (p=0.032 respectively).

A significant increase in the mean serum TNF-α was observed at follicular phase of menstrual cycle in HIV infected females on hormonal contraceptives (8.89±4.14) compared with HIV infected females not on hormonal contraceptives (3. 07±0.61) and control females on/not hormonal contraceptives (2.32±0.78, 1.30±0.63) (p=0.000 respectively). At luteal phase of menstrual cycle, a significantly increase in mean serum TNF-α was observed in HIV infected females on contraceptives (5.93±2.48) and control females on hormonal contraceptives (6.76±9.20) when compared with control females not on hormonal contraceptive (1.78±0.36) (p=0.016, 0.013 respectively).

When the mean serum IL-2 at follicular phase of menstrual cycle was compared between test and control groups, the mean serum IL-2 was significantly lower in HIV infected females on/not hormonal contraceptives (0.38±0.24, 0.12±0.05) and control females on hormonal contraceptive (0.14±0.09) compared with control females not on hormonal contraceptives (0.80±0.08) (p=0.002 respectively). However, there was significant increase in mean serum IL-2 at follicular phase of menstrual cycle in HIV infected females on hormonal contraceptives (0.38±0.24) when compared with HIV infected females not on hormonal contraceptives (0.12±0.05) and control females on hormonal contraceptives (0.14±0.09) (p=0.002, 0.005 respectively). Contrastingly, significantly lower serum IL-2 was observed for HIV infected females on hormonal contraceptives (0.38±0.24) compared with control females not on hormonal contraceptive (0.80±0.08) (p=0.002) (Table 2).

Levels of LH (iu/ml), FSH (iu/ml), prolactin (ng/ml), progesterone (ng/ml), estradiol (pg/ml) in HIV infected females and control females on/not on contraceptive (mean ±std deviation).

A significantly higher mean serum FSH was observed in control females not on hormonal contraceptive at follicular (5.56±1.79) compared with luteal (2.63±0.84) phase of menstrual cycle (p=0.001).

However, a significant decrease was observed in mean serum progesterone value in control females not on contraceptive at follicular (6.02±0.87) compared with luteal (7.21±2.33) phase of menstrual cycle (p=0.002 respectively). When the mean serum progesterone values at follicular phase of menstrual cycle was compared between test and control groups, progesterone level was significantly lower in HIV infected females on hormonal contraceptives (4.73±1.75) compared with HIV infected females not on hormonal contraceptives (7.39±0.57) and control females on/not hormonal contraceptive (6.73±0.79, 6.02±0.87) (p=0.001 respectively). Similarly, at luteal phase of menstrual cycle, when the mean serum progesterone level was compared between test and control groups, the mean serum progesterone was significantly lower in HIV infected females on hormonal contraceptives (4.32±2.65) compared with HIV infected females who were not on hormonal

contraceptives (7.42±0.93) and control females on/not on hormonal contraceptive (7.10±4.09, 7.21±2.33) (p=0.000 respectively).

A significant decrease in the mean serum progesterone value was observed at follicular phase of menstrual cycle in HIV infected females on hormonal contraceptives (4.73±1.75) compared with HIV infected females not on hormonal contraceptives (7.39±0.57) and control females on/not on hormonal contraceptives (6.73±0.79, 6.02±0.87) (p=0.003, 0.027, 0.023 respectively). Similarly, at luteal phase of menstrual cycle, a significant decrease was observed in mean value of serum progesterone in HIV infected females on contraceptives (4.32±1.65) compared with control females on/not on hormonal contraceptive (7.42±0.93, 7.21±2.33) (p = 0.001, 0.000 respectively).

When the mean serum estradiol values at follicular phase of menstrual cycle was compared between test and control groups, the mean serum estradiol value was significantly lower in HIV infected females on hormonal contraceptives (33.19±7.68) compared with HIV infected females not on hormonal contraceptives (57.89±5.50), control females on/not on hormonal contraceptive (61.24±3.55, 62.69±4.67) (p=0.000 respectively). Similarly, at luteal phase of menstrual cycle, when the mean serum estradiol were compared between test and control groups, the mean estradiol value was significantly lower in HIV infected females on hormonal contraceptives (37.52±9.83) compared with HIV infected females not on hormonal contraceptives (58.60±4.86) and control females on/not on hormonal contraceptive (61.07±2.54, 66.06±4.08) (p=0.000 respectively).

There was significant decrease in mean serum estradiol value at follicular phase of menstrual cycle in HIV infected females on hormonal contraceptives (33.19±7.68) when compared with HIV infected females not on hormonal contraceptives (57.89±5.50) and control females on/not on hormonal contraceptives (61.24±3.55, 62.69±4.67) (p=0.000 respectively). Similarly, at luteal phase of menstrual cycle, a significant decrease in mean serum estradiol value was observed in HIV infected females on contraceptives (37.52±9.83) compared with HIV infected females not on hormonal contraceptive (58.60±4.86) and control females on/not on hormonal contraceptives (61.07±2.54, 66.06±4.08) (p=0.000 respectively). Similarly, a significant decrease in mean serum estradiol level was observed in HIV infected females not on hormonal contraceptive (58.60±4.86) when compared with control females not on hormonal contraceptives (66.06±4.08) (p=0.049) (Table 3).

Correlation of BMI and blood pressure with female reproductive hormones and cytokines in HIV infected females and control females on/not on contraceptives.

Contraceptive use showed a strong positive correlation with age in HIV seronegative females on hormonal contraceptives (r=0.661, p=0.002).

Body mass index showed moderate negative correlation with estradiol in control females on hormonal contraceptives (r= -0.477, p=0.034).

Similarly, progesterone showed a moderate negative correlation with DBP in HIV infected females who were not on hormonal contraceptives (r= -0.435, p=0.030).

Similarly, SBP also showed moderate positive correlation with IL-2 (r=0.422, p=0.036), TNF-α (r=0.479, p=0.015) in HIV infected females on hormonal contraceptives and a strong negative correlation with IL-2 in HIV infected females who were not on hormonal contraceptive(r=-0.481, p=0.015) (Table 4).

Table 1: Levels of TNF α, and IL-2 in HIV infected females and control females on/not on hormonal contraceptive (mean ±std deviation)

	TNFa (pg/ml)			IL-2 (ng/ml)		
Group	**Follicular**	**Luteal**	**P- value**	**Follicular**	**Luteal**	**P - value**
A	8.89±4.14	5.93±2.48	0.963	0.38±0.24	0.23±0.10	0.145
B	3.07±0.61	3.82±1.22	0.125	0.12±0.05	0.17±0.07	0.096
C	2.32±0.78	6.76±9.20	0.225	0.14±0.09	0.76±1.31	0.234
D	1.30±0.63	1.78±0.36	0.100	0.80±0.08	0.14±0.05	0.107
F - value	14.518	2.239		6.301	2.429	
P - value	0.000	0.032		0.002	0.082	
A vs B	0.000	0.171		0.002	0.749	
A vs C	0.000	0.678		0.005	0.052	
A vs D	0.000	0.016		0.002	0.654	
B vs C	0.576	0.109		0.827	0.068	
B vs D	0.266	0.155		0.618	0.859	
C vs D	0.525	0.013		0.499	0.058	

Key: A = HIV infected females on hormonal contraceptive (n =29), B = HIV infected females not on hormonal contraceptive (n =29), C = HIV seronegative females on hormonal contraceptive (n =30), D = HIV seronegative females not on hormonal contraceptive (n =30).

Table 2: Levels of LH, FSH, Prolactin, progesterone, estradiol in HIV infected females and control females on/not on hormonal contraceptive (mean±std deviation)

	LH (iu/ml)			FSH (iu/ml)			Prolactin (ng/ml)			Progestrone(ng/ml)			Estradiol (pg/ml)		
Group	**follicular**	**luteal**	**p-value**	**follicular**	**luteal**	**p-value**	**Follicular**	**luteal**	**p-value**	**follicular**	**luteal**	**p-value**	**follicular**	**luteal**	**p-value**
A	15.33±29.93	5.91±7.36	0.400	27.58±68.94	8.10±7.35	0.407	15.51±13.13	11.85±6.48	0.483	4.73±1.75	4.32±1.65	0.724	33.19±3.68	37.52±7.83	0.568
B	16.15±13.38	8.12±10.65	0.130	10.18±3.76	11.18±19.79	0.891	17.66±18.38	18.27±14.34	0.931	7.39±0.57	7.42±3.93	0.940	57.89±5.50	58.60±4.86	0.753
C	6.25±3.05	3.52±3.00	0.155	9.58±4.85	5.14±3.52	0.113	15.92±15.93	17.38±18.58	0.887	6.73±0.79	7.10±1.09	0.514	61.24±3.55	61.07±2.54	0.931
D	4.28±4.06	7.68±13.37	0.594	5.56±1.79	2.63±0.84	0.001	17.64±17.80	26.13±20.15	0.440	6.02±0.87	7.21±2.33	0.002	62.69±4.67	66.06±4.08	0.174
F-value	0.709	0.293		0.566	0.902		0.038	1.294		4.24	12.55		20.88	16.44	
p-value	0.555	0.830		0.643	0.450		0.990	0.292		0.001	0.000		0.000	0.000	
A vs B	0.928	0.628		0.340	0.597		0.780	0.352		0.003	0.000		0.000	0.000	
A vs C	0.343	0.687		0.342	0.696		0.958	0.537		0.027	0.001		0.000	0.000	
A vs D	0.300	0.720		0.297	0.388		0.811	0.061		0.023	0.000		0.000	0.000	
B vs C	0.325	0.394		0.976	0.382		0.836	0.912		0.468	0.664		0.475	0.598	
B vs D	0.285	0.917		0.831	0.122		0.998	0.224		0.709	0.179		0.353	0.049	
C vs D	0.861	0.466		0.857	0.730		0.857	0.311		0.779	0.160		0.782	0.317	

Key: A = HIV infected females on hormonal contraceptive (n =29), B = HIV infected females not on hormonal contraceptive (n =29), C = HIV seronegative females on hormonal contraceptive (n =30), D = HIV seronegative females not on hormonal contraceptive (n =30).

Table 3: Correlation of BMI and blood pressure with female reproductive hormones, cytokines and immunoglobulin in HIV infected and control females on/not on contraceptive

Parameters	HIV infected females on contraceptives (n=29)		HIVinfected females not on contraceptives (n=29)		HIV seronegative females on contraceptives (n=30)	
	r	p	r	p	r	p
Contraceptive use vs age	-	-	-	-	0.661	0.002
BMI vs estradiol	-	-	-	-	-0.477	0.034
DBP vs prog	-	-	-0.435	0.030	-	-
IL-2 vs SBP	0.422	0.036	-	-	-	-
TNFα vs SBP	0.479	0.015	0.481	0.015	-	-

Discussion

Since the introduction of HAART, the living conditions of HIV infected persons had greatly improved; including sexuality, with many HIV infected persons desiring to have their biological children which has also resulted to so many unintended pregnancies that need to be checked with contraception to reduce; the spread of HIV infection, birth of HIV infected children and other sexually transmitted diseases. Studies have shown that HIV infected females use hormonal contraceptives less frequently than HIV seronegative females despite their effectiveness and potential benefits. [17] In this study: the effects of hormonal contraceptive on some reproductive hormones, immunoglobulin and cytokines in HIV infected females in Nnewi, Nigeria were evaluated.

This study found no significant difference in the mean serum levels of FSH, LH, prolactin, progesterone and estradiol in HIV infected females on/not on hormonal contraceptives and control females on hormonal contraceptives at both follicular and luteal phases of menstrual cycle. This contrasts the observation of elevated level of FSH at follicular phase and progesterone at luteal phase of menstrual cycle in control females not on hormonal contraceptive. In apparently healthy females, the values of estrogen, FSH and LH are usually higher at the follicular and peak at mid follicular phase to enable ovulation. [18] On the other hand, progesterone predominates at the luteal phase in association with LH; estrogen similarly rose at the mid-luteal phase thus making progesterone, LH and estrogen the hormones of luteal phase in healthy females. The absence of this pattern in HIV infected females on/not on hormonal contraceptives and control females on hormonal contraceptives may cause changes in menstrual cycle which may likely affect reproductive functions in the affected females. In apparently healthy females, FSH together with LH stimulates follicular development and maturation. The stimulated follicle produces estrogen which causes the surge in LH that induces ovulation and subsequent priming of the slugged endometrium. Following ovulation, the empty follicle luteinizes and becomes an endocrine gland that produces progesterone with the aid of LH to prepare the endometrium for implantation and sustenance of pregnancy should pregnancy occur. Otherwise, the endometrium regresses and menstruation ensues. Therefore, significant reduction in estrogen at follicular phase may cause inadequate priming of the uterus and breast and this may affect implantation, lactation and menstruation. Similarly, significant reduction of progesterone at luteal phase may affect the sustenance of pregnancy which may lead to spontaneous abortion. While significant reduction in progesterone and estrogen (hypogonadism) may

cause significant disturbances in menstrual cycle with resultant fertility challenges in the affected females. The significant decrease in the serum level of progesterone and estrogen at both follicular and luteal phases of menstrual cycle observed in HIV infected females on hormonal contraceptives when compared with HIV infected females not on hormonal contraceptives, control females on/not on hormonal contraceptives indicates presence of hypogonadism in HIV infected individuals on hormonal contraceptives. The hypogonadal effect of HIV infection was further shown with significantly reduced level of estradiol in HIV infected females not on hormonal contraceptives compared with control females not on hormonal contraceptive at luteal phase of menstrual cycle. The observation of hypogonadism in HIV infection in this study is in agreement with the works of Ikechebelu *et al.*, [4] Fallahian *et al.*, [5] and Ukibe *et al.,* [6] The hypogonadism observed in HIV infected females on hormonal contraceptive may be as a result of the combined effect of HIV infection and hormonal contraceptive on the gonads. The hormonal variation induced by HIV infection and hormonal contraceptives use may explain the high incidence of menstrual irregularities associated with hormonal contraceptive use and increased infertility associated with HIV infection as previously documented.

Studies have shown that sex hormones play a vital role in immune regulation due to the presence its receptors on the immune cells and through these receptors it interacts with cytokines to cause cytokine release. Cytokines in turn determines proliferation, maturation and differentiation of various immune cells which may result to inflammation as observed in HIV infection and hormonal contraceptive use. [19] The hypogonadism observed in HIV infected females on hormonal contraceptive could be associated with the ovarian regulatory effect of cytokines as documented by Bornstein *et al*., [20] and Lanchil *et al.,* [21] In HIV infection as with every systemic infection there are increased inflammation which are characterized by high levels of inflammatory cytokines (TNF) and low level of anti-inflammatory cytokines (IL-2) which inhibits ovarian function. [22, 23, 24] This cytokine changes were equally observed in this study. Similarly, hormonal contraceptives are known immunomodulator [3, 25] hence the altered immune response and hypogonadism observed with hormonal contraceptive use in this study. The link between hypogonadism and inflammation documented by previous author [20, 21] could be related to increase levels of inflammatory marker (TNF) observed at both follicular and luteal phases of menstrual cycles in HIV infected females on hormonal contraceptives. This explains the pronounced effect of hormonal contraceptive and HIV infection on female reproductive system as observed in HIV infected females on hormonal contraceptives in this study. Several authors [6, 26] reported that HIV infection significantly affects ovarian function in women; others [27, 28] reported no significant effect on ovarian function. This study however found that hormonal contraceptive use in HIV infection produced significant effect on ovarian functions possibly through inflammatory process.

This research observed significantly increased level of TNF-α in HIV infected females individuals on/not on hormonal contraceptives and control females on hormonal contraceptive compared with control females not on hormonal contraceptive at both follicular and luteal phases of menstrual cycle. This showed higher degree of inflammation in HIV infected females and hormonal contraceptive users when compared with control females who were not on hormonal contraceptive. Excessive TNF-α has been linked with inflammation, other ailments and ovarian failure. [24, 29] The elevated level of TnF-α observed may account for the inflammation and hypogonadism in HIV infected females on hormonal contraceptives. Similarly, the increased level of TnF-α observed in hormonal contraceptive users may be implicated in the increased risk of cancers of the breast, cervix and ovary. [30] It is well documented that HIV infection is associated with over-expression of TNF at all stages of the infection with increased viral load and depletion of CD4+ T-cells. [6] Cytokines are implicated in ovarian development and atresia, ovulation, steroidogenesis as well as in the formation, development and regression of corpus luteum. Dysregulation of these cytokines causes reduction in ovarian function and cancer of the ovary. [23, 24, 30] Hence, the hypogonadism associated with elevated level

of TNF in HIV infected hormonal contraceptive users evidenced by low levels of estradiol and progesterone as observed in this study**.** Thus the elevated levels of TNF observed in HIV infected females and hormonal contraceptive users in this study may be responsible for the inflammation and hypogonadism associated with hormonal contraceptive use in HIV infection. Similarly, the elevated levels of TNF observed in control females on hormonal contraceptive compared with control females who were not on hormonal contraceptive at luteal phase of menstrual cycle suggest that hormonal contraceptive is associated with inflammatory reaction. This showed that the combined effects of hormonal contraceptive and HIV infection may be responsible for the pronounced increase in TNF observed in HIV infected females on hormonal contraceptive as well as the hypogonadism observed in this study group. The finding of elevated TNF-α in HIV infection from this study is in tandem with the work of Enrique *et al*., [31] and Ukibe *et al*., [6]

Hormonal contraceptives and ARDs interactions can significantly affect the potency of hormonal contraceptives and ARD in HIV infection. It is documented that hormonal contraceptives especially those containing progesterone selectively compromises antiviral activity of Tenofavir and Tenofavir-alafenamide [32] and this may suggests decrease ARD protection in hormonal contraceptive users. Similarly, Efavirenz and Nevirapine significantly reduce the effectiveness of progesterone containing contraceptive [17, 33, 34] thus resulting to contraceptive failures. The interaction of hormonal contraceptives with ARD may account for the pronounced inflammation observed in HIV infected females on hormonal contraceptives in this study.

This study observed significantly decreased levels of IL-2 in HIV infected females on/not on hormonal contraceptive and control females on hormonal contraceptive compared with control females not on hormonal contraceptive at follicular phase of menstrual cycle, these suggest a depressed immunity in HIV infection and hormonal contraceptive use at follicular phase of menstrual cycle in these study groups, this observation is in agreement with decreased IL-2 in HIV infection and hormonal contraceptive as recorded by Stringer *et al.,* [3] The follicular phase of menstrual cycle (associated with high level of FSH, LH, estrogen and low level of progesterone) coincides with the period of egg development and menstrual bleeding. At this phase, there is less susceptibility to infection due to increased immune response attributed to estrogen that helps to protect the embryo should fertilization occur. However, the luteal phase is associated with depressed immunity and the reduced inflammatory response predisposes to increased susceptibility to infection and it coincides with the period of high level of progesterone in apparently healthy females, [35, 36] the suppressed immune response is vital to accommodate the fertilized embryo should conception occur. The cyclical changes of menstrual cycle caused by changing levels of female sex hormones such as estrogen and progesterone influence the female immune system thus predisposing to increased or reduced risk of infection at different phases of menstrual cycle. This pattern was not observed in HIV infected females on hormonal contraceptive, HIV infected females who were not on hormonal contraceptive and control females on hormonal contraceptive. These observations suggest that HIV infection and hormonal contraceptives alters the immune response to microorganism.

BMI showed moderate negative correlation with estradiol in control females on hormonal contraceptives. This indicates that with increasing BMI in hormonal contraceptive users there is high tendency to decreased level of gonadal (estradiol) hormone which suggest that prolonged contraceptive use associated with raised BMI may predispose the user of hormonal contraceptive to hypogonadism as reflected with the negative correlation in this study. BMI is used to measure body fitness and extreme BMI are associated with reproductive challenges such as anovulation, menstrual irregularities, PCOS, infertility etc. Therefore, raised BMI (obesity) disrupts the hypothalamic-pituitary gonadal axis thus affecting the reproductive health of females. Several studies have established that obesity has a direct link with infertility as manifested by low levels of gonadal hormones. [37, 38]

Studies have shown that cytokines can alter the hemodynamic parameters by binding to angiotensin II type 1 receptors thereby activating T-lymphocyte and

release of proinflammatory cytokine (such as TNF) which may provoke inflammatory response in the vasculature thus causing alterations in blood pressure which overtime may lead to cardiovascular diseases such as atherosclerosis. [39, 40] This study showed moderate negative correlation between DBP and progesterone. The relationship between progesterone and blood pressure is controversial. While some authors associate increase level of progesterone with high blood pressure. [41, 42, 43] Kristianson *et al.,* [43] reported that progesterone lowers blood pressure by binding to mineralocorticoid receptors thus inhibiting aldosterone activity with resultant natriuresis. However, in females with gain-of-function mineralocorticoid receptor mutation; progesterone increases blood pressure and this mutation may be responsible for the sudden death in young women on fourth generation hormonal contraceptive (drosperinone).

Hormonal contraceptive use showed strong positive correlated with age in control females on hormonal contraceptive. Age is an important risk factor for cardiovascular diseases, in healthy females at younger age; there is lower risk of CVD due to protective effect of estrogen but at menopause, the levels of estrogen falls which then predispose women to increased risk of cardiovascular diseases [44, 45] The observation in this study is similar to that in apparently healthy premenopausal females. As the duration of contraceptives increases with age the risk of CVD increases, this may be the reason why as the age of a woman advances an alternate choice of contraceptives are considered.

Conclusion

The decreased level of ovarian hormones (estradiol and progesterone) observed at both phases of menstrual cycle suggests hypogonadism while the changes in the inflammatory markers may result to active inflammation and suppressed immunity. The profound inflammatory impact observed may possibly be due to combined effects of hormonal contraceptives and HIV infection. HIV infected females on hormonal contraceptives should therefore, be closely monitored for inflammation and reproductive changes through routine screening and reproductive education irrespective of contraceptive use. The drug interaction between ARD and hormonal contraceptive should be critically examined before placing a patient on a particular contraception method.

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